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(54) **MUCOSAL VASCULAR ADDRESSINS AND USES THEREOF**

ADRESSINE DER SCHLEIMHAUT UND DER BLUTGEFÄSSE UND IHRE VERWENDUNG
ADRESSINES VASCULAIRES DE MUQUEUSES ET LEURS UTILISATIONS

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- **JOURNAL OF IMMUNOLOGY**, vol. 153, no. 1, 1 July 1994, BALTIMORE US, pages 517-527, XP002006075 D.J.ERLE ET AL.: "Expression and function of the MAdCAM-1 receptor, integrin alpha4beta7, on human leukocytes"
- **NATURE**, vol. 363, 3 June 1993, LONDON GB, pages 461-464, XP002006076 M.J.BRISKIN ET AL.: "MAdCAM-1 has homology to immunoglobulin and mucin-like adhesion receptors and to IgA1"
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- **HESTERBERG P.E. ET AL.**: 'Rapid resolution of chronic colitis in the cotton-top tamarin with an antibody to a gut-homing integrin alpha4beta7' **GASTROENTEROLOGY** vol. 111, 1996, pages 1373 - 1380
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Description

Background of the Invention

[0001] Lymphocyte homing from the circulation to the lymphoid tissues and migration to sites of inflammation is regulated by interaction with receptors expressed in postcapillary venules, including high endothelial venules (HEV) found in secondary lymphoid tissues (e.g., mesenteric lymph nodes, Peyer's Patches (PP)) (Bevilacqua, M.P., *Annu. Rev. Immunol.*, 11: 767-804 (1993); Butcher, E.C., *Cell*, 67: 1033-1036 (1991); Picker, L.J., et al., *Annu. Rev. Immunol.*, 10: 561-591 (1992); and Springer, T.A., *Cell*, 76: 301-314 (1994)). These interactions are tissue specific in nature.

[0002] Inflammation (e.g., chronic inflammation) is characterized by infiltration of the affected tissue by leukocytes, such as lymphocytes, lymphoblasts, and mononuclear phagocytes. The remarkable selectivity by which leukocytes preferentially migrate to various tissues during both normal circulation and inflammation results from a series of adhesive and activating events involving multiple receptor-ligand interactions as proposed by Butcher and others (Butcher, E.C., *Cell*, 67: 1033-1036 (1991); vonAdrian, U.H., et al., *Proc. Natl. Acad. Sci. USA*, 88: 7538 (1991); Mayadas, T.N., et al., *Cell*, 74: 541 (1993); Springer, T.A., *Cell*, 76: 301 (1994)). As an initial step, there is a transient, rolling interaction between leukocytes and endothelium, which results from the interaction of selectins (and by $\alpha 4$ integrins in some instances) with their carbohydrate ligands. This interaction which is characterized by rolling in the direction of flow can be assessed by known methods (Lawrence, M.B. and T.A. Springer, *Cell*, 65: 859 (1991); WO 92/21746, Springer *et al.*, (December 10, 1992)). This is followed by activation events mediated by chemoattractants such as chemokines and their receptors, which cause activation of integrin adhesiveness and influence the direction of migration of leukocytes through vascular walls. Such secondary signals in turn trigger the firm adhesion of leukocytes to endothelium via leukocyte integrins and their endothelial ligands (Ig-like receptors and the ECM), and subsequent transendothelial migration from the circulation across the vascular endothelium.

[0003] In secondary lymphoid tissues, such as Peyer's patches (PPs) and lymph nodes (e.g., peripheral lymph nodes (PLN)), leukocyte trafficking and homing is regulated by interactions of homing receptors on the surface of leukocytes with endothelial cells lining the post-capillary venules, notably high endothelial venules (HEV) (Gowans, J.L. and E.J. Knight, *Proc. R. Soc. Lond.*, 159: 257 (1964)). Receptors termed vascular addressins, which are present on the endothelial cell surface and regulate the migration and subsequent extravasation of lymphocyte subsets. The vascular addressins show restricted patterns of expression and this tissue specific expression makes an important contribution to the specificity of leukocyte trafficking (Picker, L.J. and E.C. Butcher, *Annu. Rev. Immunol.*, 10: 561-591 (1992); Berg, E.L., et al., *Cellular and molecular mechanisms of inflammation*, 2: 111 (1991); Butcher, E.C., *Cell*, 67: 1033-1036 (1991)).

[0004] Mucosal vascular addressin MAdCAM-1 (Mucosal Addressin Cell Adhesion Molecule-1) is an immunoglobulin superfamily adhesion receptor for lymphocytes, which is distinct from VCAM-1 and ICAM-1. MAdCAM-1 was identified in the mouse as a -60 kd glycoprotein which is selectively expressed at sites of lymphocyte extravasation. In particular, MAdCAM-1 expression was reported in vascular endothelial cells of mucosal tissues, including gut-associated tissues or lymphoid organs, such as Peyer's patches and venules of the lamina propria of the small and large intestine, and the lactating mammary gland, but not in peripheral lymph nodes. MAdCAM-1 is involved in lymphocyte binding to Peyer's Patches. (Streeter, P.R., et al., *Nature*, 331: 41-46 (1988); Nakache, M., et al., *Nature*, 337: 179-181 (1989); Picker, L.J., et al., *Annu. Rev. Immunol.*, 10: 561-591 (1992); Briskin, M.J., et al., *Nature*, 363: 461 (1993); Berg, E.L., et al., *Nature*, 366: 695-698 (1993); Berlin, C., et al., *Cell*, 74: 185-195 (1993)). MAdCAM-1 can be induced *in vitro* by proinflammatory stimuli (Sikorski, E.E., et al., *J. Immunol.*, 151: 5239-5250 (1993)).

[0005] MAdCAM-1 specifically binds the lymphocyte integrin $\alpha 4 \beta 7$ (also referred to as LPAM-1 (mouse), $\alpha 4 \beta p$ (mouse)), which is a lymphocyte homing receptor involved in homing to Peyer's patches (Berlin, C., et al., *Cell*, 80: 413-422 (1994); Berlin, C., et al., *Cell*, 74: 185-195 (1993); and Erle, D.J., et al., *J. Immunol.*, 153: 517-528 (1994)). In contrast to VCAM-1 and fibronectin, which interact with both $\alpha 4 \beta 1$ and $\alpha 4 \beta 7$ (Berlin, C., et al., *Cell*, 74: 185-195 (1993); Strauch, U.S., et al., *Int. Immunol.*, 6: 263 (1994)), MAdCAM-1 is a selective receptor for $\alpha 4 \beta 7$.

[0006] Inflammatory bowel disease (IBD), such as ulcerative colitis and Crohn's disease, for example, can be a debilitating and progressive disease involving inflammation of the gastrointestinal tract. Affecting an estimated two million people in the United States alone, symptoms include abdominal pain, cramping, diarrhea and rectal bleeding. IBD treatments have included anti-inflammatory drugs (such as, corticosteroids and sulfasalazine), immunosuppressive drugs (such as, 6-mercaptopurine, cyclosporine and azathioprine) and surgery (such as, colectomy). Podolsky, *New Engl. J. Med.*, 325: 928-937 (1991) and Podolsky, *New Engl. J. Med.*, 325: 1008-1016 (1991).

[0007] Some studies have suggested that the cell adhesion molecule, ICAM-1, mediates leukocyte recruitment to inflammatory sites through adhesion to leukocyte surface ligands, i.e., Mac-1 or LFA-1 (Springer, *Nature*, 346: 425-434 (1990)). In addition, vascular cell adhesion molecule-1 (VCAM-1), which recognizes the $\alpha 4 \beta 1$ integrin (VLA-4), has been reported to play a role in *in vivo* leukocyte recruitment (Silber et al., *J. Clin. Invest.* 93: 1554-1563 (1994)). It has been proposed that IBD can be treated by blocking the interaction of ICAM-1 with LFA-1 or Mac-1, or of VCAM-1 with $\alpha 4 \beta 1$ (e.g., WO 93/15764). However, these therapeutic targets are likely to be involved in inflammatory processes in multiple

organs, and a functional blockade could cause systemic immune dysfunction.

[0008] In contrast to VCAM-1 and ICAM-1, MAdCAM is preferentially expressed in the gastrointestinal tract, binds the $\alpha 4\beta 7$ integrin found on lymphocytes, and participates in the homing of these cells to mucosal sites, such as Peyer's patches in the intestinal wall (Hamann et al., Journal of Immunology, 152: 3282-3293 (1994)). The use of inhibitors to the binding of MAdCAM to the receptor, $\alpha 4\beta 7$, in the treatment of diseases such as IBD has not been suggested. Moreover, although human $\alpha 4$ and $\beta 7$ genes and proteins have been identified (Yuan et al., Int. Immunol., 2: 1097-1108 (1990); Erle et al., J. Biol. Chem., 266: 11009-11016 (1991); Bevilacqua, M.P., Annu. Rev. Immunol., 11: 767-804 (1993); Springer, T.A., Cell, 76: 301-314 (1994)), human or primate MAdCAM-1 has not been cloned or characterized.

[0009] WO 94/13312 is directed to the provision of mammalian purified nucleic acids and proteins encoding mucosal addressins. The proteins, nucleic acids and fragments thereof serve a variety of purposes in modulating leukocyte mucosal tissue homing interactions. In addition the protein fragments may serve as antigens to produce antibodies specific for particular domains of the mucosal addressin, while the nucleic acids may be used as probes, for antisense preparation for introduction into hosts which may be allogenic or xenogeneic to the DNA source, for expression of the protein and the like.

Summary of the Invention

[0010] In one aspect the invention relates to an isolated nucleic acid which encodes

- (a) a naturally occurring primate MAdCAM, or
- (b) a functional variant thereof which binds $\alpha 4\beta 7$ integrin; or
- (c) a functional fragment of a naturally occurring primate MAdCAM which binds $\alpha 4\beta 7$ integrin and comprises at least one immunoglobulin-like domain,

wherein the amino acid sequence of said functional variant is at least 75% similar to the sequence of a protein shown in Figure 1 (SEQ ID NO:2), Figure 2 (SEQ ID NO:4) or Figure 3 (SEQ ID NO:6), and amino acid sequence similarity is determined by the Clustal method with the PAM250 residue weight table, using a gap penalty of 10, a gap length penalty of 10 and pairwise alignment parameters of ktuple = 1, gap penalty = 3, window = 4, and diagonals saved = 5.

[0011] In another aspect the invention relates to an isolated protein which has the amino acid sequence of

- a) a naturally occurring primate MAdCAM, or
- (b) a functional variant thereof which binds $\alpha 4\beta 7$ integrin; or
- (c) a functional fragment of a naturally occurring primate MAdCAM which binds $\alpha 4\beta 7$ integrin and comprises at least one immunoglobulin-like domain,

wherein the amino acid sequence of said functional variant is at least 75% similar to the sequence of a protein shown in Figure 1 (SEQ ID NO:2), Figure 2 (SEQ ID NO:4) or Figure 3 (SEQ ID NO:6), and amino acid sequence similarity is determined by the Clustal method with the PAM250 residue weight table, using a gap penalty of 10, a gap length penalty of 10 and pairwise alignment parameters of ktuple = 1, gap penalty = 3, window = 4, and diagonals saved = 5.

[0012] In a further aspect the invention relates to an isolated naturally occurring primate MAdCAM having one or more functions selected from the group consisting of binding to $\alpha 4\beta 7$ integrin and mediation of cellular adhesion.

[0013] In a further aspect the invention relates to a method of producing a protein of the invention comprising maintaining a host cell containing recombinant nucleic acid encoding said protein under conditions suitable for expression of the nucleic acid, whereby said protein is produced.

[0014] In a further aspect the invention relates to an antibody or antigen-binding fragment thereof which binds a naturally occurring primate MAdCAM.

[0015] In a further aspect the invention relates to an antibody or antigen-binding fragment of the invention for use in therapy or diagnosis.

[0016] In a further aspect the invention relates to the use of an antibody or antigen-binding fragment of the invention for the manufacture of a medicament.

[0017] In a further aspect the invention relates to a method of detecting a selected primate MAdCAM in a sample comprising:

- (a) contacting a sample with an antibody or antigen-binding fragment thereof which binds a naturally occurring primate MAdCAM under conditions suitable for specific binding of said antibody or fragment thereto; and
- (b) detecting the formation of a complex between said antibody or fragment and MAdCAM.

[0018] In a further aspect the invention relates to a method of detecting or identifying a ligand of or an agent which

binds a primate MAdCAM comprising combining an agent to be tested with an isolated protein of the invention under conditions suitable for binding of ligand thereto, and detecting or measuring the formation of a complex between said agent and said protein.

[0019] In a further aspect the invention relates to a method of detecting an inhibitor of binding of a naturally occurring primate MAdCAM to a ligand thereof comprising:

- (a) combining an agent to be tested with a ligand of said primate MAdCAM and a composition comprising isolated and/or recombinant primate MAdCAM of the invention under conditions suitable for binding of ligand thereto; and
- (b) detecting or measuring binding between primate MAdCAM and ligand, whereby decreased binding as compared with a suitable control indicates that the agent is an inhibitor.

[0020] The present invention relates to proteins or polypeptides, referred to herein as isolated and/or recombinant (e.g., essentially pure) primate MAdCAMs. In one embodiment, primate MAdCAM can selectively bind to cells which express the $\alpha 4\beta 7$ integrin, particularly lymphocytes. The recombinant proteins of the present invention, including variants, can be produced in host cells as described herein. In addition, antibodies reactive with the proteins of the present invention can be produced using a primate MAdCAM or a variant thereof as immunogen, for example. Such antibodies or fragments thereof are useful in therapeutic, diagnostic and research applications. For example the antibodies can be used in the purification and study of MAdCAMs, the identification of cells which express MAdCAM and the detection or quantitation of MAdCAM in a sample.

[0021] The invention further relates to isolated and/or recombinant (e.g., essentially pure) nucleic acids which encode a primate MAdCAM, such as human MAdCAMs. In another aspect, the invention relates to recombinant nucleic acid constructs, such as plasmids or retroviral vectors, which contain a nucleic acid which encodes a protein of the present invention or portion thereof. The nucleic acids and constructs can be used to produce recombinant primate MAdCAMs. In another embodiment, the nucleic acid encodes an antisense nucleic acid which can hybridize with a second nucleic acid encoding a primate MAdCAM and which can inhibit the expression of the protein (e.g., when introduced into cells).

[0022] Also encompassed by the present invention are methods of identifying ligands and/or inhibitors (e.g., antagonists) of MAdCAM function. For example, primate MAdCAM, including variants, can be used in assays (e.g., adhesion assays) designed to identify antagonists which block the binding of MAdCAM to the ligand, $\alpha 4\beta 7$ integrin.

[0023] The invention further relates to methods of therapy, including a method of treating an individual suffering from a disease associated with leukocyte (such as lymphocyte or monocyte) recruitment to the gastrointestinal tract or other tissues as a result of binding of leukocytes to gut-associated endothelium expressing the molecule MAdCAM, comprising administering to the individual (e.g., a mammal, such as a primate) an effective amount of an agent or compound, such as an antibody, which inhibits the binding of leukocytes to endothelial MAdCAM. The antibody is preferably a monoclonal, chimeric and/or humanized antibody or an antigen binding fragment thereof, and inhibits adhesion of leukocytes expressing an integrin containing the $\beta 7$ chain (such as $\alpha 4\beta 7$) to endothelium expressing MAdCAM. In one embodiment, the monoclonal antibody or antigen binding fragment thereof has the antigenic specificity of a monoclonal antibody selected from the group consisting of FIB 21, FIB 30, FIB 504 and ACT-1. Inflammatory bowel diseases, such as, but not limited to, ulcerative colitis, Crohn's disease, Pouchitis, celiac disease, microscopic or collagenous colitis, and eosinophilic gastroenteritis can be treated according to the claimed method.

Brief Description of the Drawings

[0024]

Figure 1 is an illustration of the nucleotide sequence (SEQ ID NO:1) determined from subclones of cDNA clone 4 encoding human MAdCAM-1, and the sequence of the predicted protein encoded by the open reading frame (MAdCAM-1; SEQ ID NO:2). The predicted signal peptide and transmembrane region are underlined in bold. Cysteine residues of the two Ig-like domains are boxed, as are potential N-linked glycosylation sites. The mucin domain, containing the PPDTTS(Q/P)E repeat consisting of 71 amino acids is outlined by a thin bold line.

Figure 2 is an illustration of the nucleotide sequence (SEQ ID NO:3) determined from subclones of cDNA clone 20 encoding human MAdCAM-1, and the sequence of the predicted protein encoded by the open reading frame (MAdCAM-1; SEQ ID NO:4). The predicted signal peptide and transmembrane region are underlined in bold. Cysteine residues of the two Ig-like domains are boxed, as are potential N-linked glycosylation sites. The mucin domain, containing the PPDTTS(Q/P)E repeat consisting of 47 amino acids is outlined by a thin bold line.

Figure 3 is an illustration of the nucleotide sequence (SEQ ID NO:5) determined from subclones of cDNA clone 31D encoding macaque MAdCAM-1, and the sequence of the predicted protein encoded by the open reading frame (MAdCAM-1; SEQ ID NO:6). The predicted signal peptide and transmembrane region are underlined in bold. Cysteine residues of the two Ig-like domains are boxed. The mucin domain, which contains a single PPDTTS(Q/P)E repeat,

is outlined by a thin bold line.

Figures 4A-4B are histograms illustrating the selective binding of cells transfected with human MAdCAM-1 to lymphocytes expressing $\alpha 4\beta 7$. Figure 4A illustrates the results of an experiment in which RPMI 8866 cells (0.5×10^6 /well), which express $\alpha 4\beta 7$ (and not $\alpha 4\beta 1$), bound to CHO/P cells expressing murine or human MAdCAM-1, but did not bind to CHO/P cells transfected with human VCAM-1 or to CHO/P cells transfected with pcDNA-3. Figure 4B illustrates the results of an experiment in which CHO/P cells transfected with human VCAM-1 bound to Jurkat cells (which express high levels of $\alpha 4\beta 1$), but failed to bind to CHO/P cells transfected with murine or human MAdCAM-1 or to CHO/P cells transfected with pcDNA-3 as a control. Binding is shown as the number of bound RPMI 8866 cells per CHO/P cell (Figure 4A) or bound Jurkat cells per CHO/P cell (Figure 4B) in an average of at least four fields (10X objective) $\pm$ standard error. Binding reactions included control IgG, anti- $\alpha 4\beta 7$ (monoclonal antibody ACT-1), or anti-murine MAdCAM-1 (monoclonal antibody MECA-367) as indicated.

Figure 5 is a histogram illustrating that human MAdCAM-1 encoded by clones 4 and 20 binds RPMI 8866 cells and that binding is inhibited by the ACT-1 antibody. Bars represent an average of four fields from a single experiment with standard deviations as shown.

Figure 6 is an illustration of the deduced domain structures of murine and human MAdCAM-1. The two N-terminal immunoglobulin domains bounded by disulfide bonds (indicated by loops) implicated in cell adhesion, transmembrane regions and a cytoplasmic tail are present in murine, macaque and human proteins. Human MAdCAM-1 has a longer cytoplasmic tail. An eight-amino acid repeat found in the mucin domain is present in 4 or 8 copies in human isoforms, but appears only once in the murine and macaque.

Figures 7A and 7B are graphic illustrations of histologic scores of inflammatory activity and epithelial injury from left (descending) and right (ascending) colon of mice exposed to 10 days of DSS in their drinking water. Three groups of mice are shown, consisting of groups receiving an irrelevant rat IgG2a antibody, FIB 21, or FIB 30 antibodies.

Figure 8 is a graph of γ counts per minute (cpm) (± 1 SEM) as a percentage of total input counts from mice given DSS in the drinking water for 10 days. Six groups consisted of negative controls given water alone, positive controls given DSS alone, test groups given irrelevant rat IgG2a antibody, FIB21, MECA-367, or FIB21 with MECA-367.

Figure 9 is a graph depicting the histologic scores (± 1 SEM) for villus fusion obtained from jejunal biopsy samples of common marmosets before and on the 14th day of treatment with 2 mg/kg/day of ACT-1 monoclonal antibody.

Figure 10 is a graph depicting the histologic scores (± 1 SEM) for villus atrophy obtained from jejunal biopsy samples of common marmosets before and on the 14th day of treatment with 2 mg/kg/day of ACT-1 monoclonal antibody.

Figure 11 is an illustration of the stool consistency of colitic animals (cotton-top tamarins) treated with ACT-1 antibody. Figure 12 is an illustration of the inflammatory activity in colitic animals (cotton-top tamarins) treated with ACT-1 antibody as assessed histologically.

Figure 13 is a diagram illustrating the experimental protocol for treatment of chronically colitic cotton-top tamarins with ACT-1 antibody.

Figure 14 is a graph illustrating the therapeutic effect on stool consistency of administration of ACT-1 antibody (-●-) or an irrelevant, isotype-matched antibody (-O-) to chronically colitic cotton-top tamarins.

Figure 15 is a histogram illustrating the therapeutic effect of ACT-1 immunotherapy on colonic inflammatory activity in chronically colitic cotton-top tamarins treated with ACT-1 antibody or an irrelevant control monoclonal antibody. Each bar represents the mean within a treatment group of the absolute change in the inflammatory activity score ± 1 SEM, computed for each animal by comparing the score at a particular time point with the same animal's score on Day 0.

Figure 16 is a histogram illustrating the absolute numbers of $\alpha 4\beta 7^+$ lymphocytes in the peripheral circulatory pool in colitic cotton-top tamarins treated with ACT-1 antibody. Each bar represents the mean (± 1 SEM) of the concentration of $\alpha 4\beta 7^+$ cells in blood as detected by ACT-1.

Figures 17A-17E are plots illustrating the results of a FACS analysis showing the specific staining of Hut 78 cells with MAdCAM-Ig chimeras. Supernatants from COS cells transiently transfected with a human MAdCAM-Ig chimeric constructs (from four independent transfections) were incubated with HuT 78 cells in the presence of 2 mM Mn^{++} , and bound chimera was detected using a phycoerythrin-conjugated antibody specific for human IgG1. Figure 17A, media control; Figures 17B-17C, supernatants from cells transfected with Clone 21 (comprising the entire extracellular domain of human MAdCAM); Figures 17D-17E, supernatants from cells Clone 38 (comprising the two N-terminal Ig domains of human MAdCAM). Binding after preincubation of cells with media alone (at right). Binding was inhibited by preincubation of cells with anti- $\beta 7$ MAb FIB 504 (at left).

Figure 18 is a graph illustrating the difference in body weight of *scid* mice reconstituted with 1×10^6 CD45RB^{hi} T cells (■) relative to control *scid* mice reconstituted with an equal number of CD45RB^{lo} T cells (▲) derived from BALB/c spleen. Recipient mice were weighed at weekly intervals to evaluate progression of disease.

Figure 19 is a histogram illustrating the increased accumulation in the colon of intravenously injected ^{111}In -labeled mesenteric lymph node cells in *scid* mice reconstituted with CD45RB^{hi} T cells as compared with the accumulation in colon of *scid* mice reconstituted with an equal number of CD45RB^{lo} T cells, and the inhibition of accumulation by

treatment for 2 weeks with a combination of anti- $\beta 7$ (FIB 504) and anti-MAdCAM (MECA-367) monoclonal antibodies. Results are expressed as % counts per minute (CPM) in colon normalized to CPM in spleen and corrected for background.

Figure 20 is a histogram illustrating the complete inhibition accumulation of ^{111}In -labeled cells (injected intravenously) in the colon of *scid* mice by treatment for 4 months (starting from the time of reconstitution) with FIB 504, MECA-367, or FIB 504 plus MECA-367. From left to right: *scid* mice reconstituted with CD45RB^{lo} T cells, receiving irrelevant isotype-matched control rat IgG2a; *scid* mice reconstituted with CD45RB^{hi} T cells, receiving either irrelevant isotype-matched control rat IgG2a, FIB 504, MECA-367, or FIB 504 + MECA-367.

Figure 21 is a histogram illustrating the reduction in the number of CD4⁺ T cells in the ascending (right) or descending (left) colon in *scid* mice treated for 14 days with a combination of FIB 504 plus MECA-367 as compared to mice treated with an isotype-matched control rat IgG2a antibody as determined by staining frozen sections of left and right colon with a rat antibody specific for mouse CD4.

Detailed Description of the Invention

Proteins and Peptides

[0025] The present invention relates to isolated and/or recombinant (including, e.g., essentially pure) proteins or polypeptides designated primate MAdCAMs (Mucosal Addressin Cell Adhesion Molecules) and variants of primate MAdCAMs. In a preferred embodiment, the isolated and/or recombinant proteins of the present invention have at least one property, activity or function characteristic of a primate MAdCAM (as defined herein), such as binding function (e.g., the ability to bind an $\alpha 4\beta 7$ integrin), and/or cellular adhesion molecule function (e.g., the ability to mediate cellular adhesion such as $\alpha 4\beta 7$ -dependent adhesion *in vitro* and/or *in vivo*), and/or an immunological property as defined herein. For example, some proteins of the present invention can selectively bind to an $\alpha 4\beta 7$ integrin and thereby mediate $\alpha 4\beta 7$ -dependent cellular adhesion to cells bearing the $\alpha 4\beta 7$ integrin, such as leukocytes (especially lymphocytes such as T or B cells) *in vitro* and/or *in vivo*. In one aspect, proteins of the present invention can mediate heterotypic cell adhesion (e.g., of endothelial cells to leukocytes such as lymphocytes).

[0026] In another embodiment, proteins of the present invention can bind a primate $\alpha 4\beta 7$ integrin from the same or a different primate species, and/or have cellular adhesion molecule function (e.g., the ability to mediate cellular adhesion such as $\alpha 4\beta 7$ -dependent adhesion *in vitro* and/or *in vivo*). For example, as shown herein, human and macaque MAdCAM-1 proteins, produced in mammalian cells by expression of cDNA clones, can selectively bind to $\alpha 4\beta 7$ integrin present on human lymphocytes, and can function as cellular adhesion molecules capable of mediating selective adhesion to cells bearing the $\alpha 4\beta 7$ integrin.

[0027] Proteins or polypeptides referred to herein as "isolated" are proteins or polypeptides purified to a state beyond that in which they exist in mammalian cells. "Isolated" proteins or polypeptides include proteins or polypeptides obtained by methods described herein, similar methods or other suitable methods, including essentially pure proteins or polypeptides, proteins or polypeptides produced by chemical synthesis (e.g., synthetic peptides), or by combinations of biological and chemical methods, and recombinant proteins or polypeptides which are isolated. The proteins can be obtained in an isolated state of at least about 50 % by weight, preferably at least about 75 % by weight, and more preferably, in essentially pure form. Proteins or polypeptides referred to herein as "recombinant" are proteins or polypeptides produced by the expression of recombinant nucleic acids.

[0028] As used herein "primate MAdCAM" refers to naturally occurring or endogenous primate MAdCAM proteins, to proteins having an amino acid sequence which is the same as that of a naturally occurring or endogenous corresponding primate MAdCAM (e.g., recombinant proteins), and to functional variants of each of the foregoing (e.g., functional fragments and/or mutants produced via mutagenesis and/or recombinant techniques). Accordingly, as defined herein, the term includes mature primate MAdCAM, glycosylated or unglycosylated MAdCAM proteins, polymorphic or allelic variants, and other isoforms of primate MAdCAM (e.g., produced by alternative splicing or other cellular processes), and functional fragments.

[0029] Naturally occurring or endogenous primate MAdCAM proteins includes wild type proteins such as mature MAdCAM, polymorphic or allelic variants and other isoforms which occur naturally in primates (e.g., humans or other non-human primates, such as macaque, cotton top tamarin). Such proteins can be recovered from a source which naturally produces primate MAdCAM. These proteins and primate MAdCAM proteins having the same amino acid sequence as a naturally occurring or endogenous corresponding primate MAdCAM, are referred to by the name of the corresponding primate. For example, where the corresponding primate is a human, the protein is designated as a human MAdCAM protein (e.g., a recombinant human MAdCAM produced in a suitable host cell).

[0030] "Functional variants" of primate MAdCAMs include functional fragments, functional mutant proteins, and/or functional fusion proteins. Generally, fragments or portions of primate MAdCAM encompassed by the present invention include those having a deletion (i.e., one or more deletions) of an amino acid (i.e., one or more amino acids) relative to

the mature primate MAdCAM (such as N-terminal, C-terminal or internal deletions). Fragments or portions in which only contiguous amino acids have been deleted or in which non-contiguous amino acids have been deleted relative to mature primate MAdCAM are also envisioned.

[0031] Generally, mutants or derivatives of primate MAdCAMs, encompassed by the present invention include natural or artificial variants differing by the addition, deletion and/or substitution of one or more contiguous or non-contiguous amino acid residues, or modified polypeptides in which one or more residues is modified, and mutants comprising one or more modified residues. Preferred mutants are natural or artificial variants of primate MAdCAM differing by the addition, deletion and/or substitution of one or more contiguous or non-contiguous amino acid residues.

[0032] A "functional fragment or portion", "functional mutant" and/or "functional fusion protein" of a primate MAdCAM refers to an isolated and/or recombinant protein or oligopeptide which has at least one property, activity and/or function characteristic of a primate MAdCAM, such as binding function (e.g., the ability to bind an $\alpha 4\beta 7$ integrin), and/or cellular adhesion molecule function (e.g., the ability to mediate cellular adhesion such as $\alpha 4\beta 7$ -dependent adhesion *in vitro* and/or *in vivo*), and/or retains at least one immunological property of a primate MAdCAM.

[0033] As used herein, a protein, polypeptide or oligopeptide having "at least one immunological property" of a primate MAdCAM is one which (a) is bound by at least one antibody of a selected epitopic specificity which binds to a naturally occurring or endogenous primate MAdCAM or to a protein having the same amino acid sequence as the naturally occurring or endogenous primate MAdCAM (e.g., human MAdCAM-1), and/or (b) is an immunogen capable of inducing the formation in a suitable animal of an antibody of a selected epitopic specificity which binds to a naturally occurring or endogenous primate MAdCAM or to a protein having the same amino acid sequence as the naturally occurring or endogenous primate MAdCAM. For example, a suitable fragment can cross-react with an antibody which is raised against and/or reactive with isolated primate MAdCAM.

[0034] Suitable fragments or mutants can be identified by screening. For example, the N-terminal, C-terminal, or internal regions of the protein can be deleted in a stepwise fashion and the resulting protein or polypeptide can be screened using a suitable binding or adhesion assay, such as an assay described herein. Where the resulting protein displays activity in the assay, the resulting protein ("fragment") is functional. Information regarding the structure and function of murine MAdCAM and other adhesion molecules, and of primate MAdCAMs as shown herein, provides a basis for dividing primate MAdCAM into functional domains (see below).

[0035] The term variant also encompasses fusion proteins, comprising a primate MAdCAM (e.g., mature human MAdCAM-1) as a first moiety, linked to a second moiety not occurring in the primate MAdCAM as found in nature. Thus, the second moiety can be an amino acid, oligopeptide or polypeptide. The first moiety can be in an N-terminal location, C-terminal location or internal to the fusion protein. In one embodiment, the fusion protein comprises a human MAdCAM or portion thereof as the first moiety, and a second moiety comprising a linker sequence and affinity ligand (e.g., an enzyme, an antigen, epitope tag).

[0036] In another embodiment, the fusion protein is a hybrid immunoglobulin, such as a hybrid comprising a primate MAdCAM moiety fused at its c-terminus, to the N-terminus of an immunoglobulin moiety (e.g., one or more immunoglobulin constant regions, preferably of primate origin), such as those prepared according to Capon et al., U.S. Patent No. 5,428,130 and 5,225,538, the teachings of which are incorporated herein by reference in their entirety). The hybrid immunoglobulin comprises a fusion protein or polypeptide containing at least a portion of an immunoglobulin chain, and preferably at least one complete immunoglobulin domain (e.g., CH1, hinge). Other examples of "immunoadhesins" have been reported (Watson, S.R., et al., *Nature*, 349: 164-167 (1991); Martin, S., et al., *J. Virol.*, 67: 3561-3568 (1993); Staunton, D.E., et al., *J. Exp. Med.*, 176: 1471-1476 (1992); Capon, D.J., et al., *Nature*, 337: 525-531 (1989); Jakubowski et al., *J. Immunol.*, 155: 938-946 (1995)). For example, a fusion protein comprising all or a portion of a primate (e.g., human) MAdCAM and an immunoglobulin heavy or light chain constant region or portion thereof can be prepared (e.g., by preparing a nucleic acid which encodes the fusion protein). Typically, the fusion is constructed such that the C-terminal end of the MAdCAM is joined to the N-terminal end of the immunoglobulin constant region. However, fusion proteins in which the N-terminal end of the MAdCAM is joined to the C-terminal end of the immunoglobulin constant region can be made. Preferably, a portion of primate MAdCAM which is sufficient for binding to a ligand (e.g., $\alpha 4\beta 7$ integrin), such as the complete extracellular domain or a portion comprising the two N-terminal immunoglobulin domains in which the transmembrane region is deleted, is used (see e.g., Example 3). -

[0037] A variety of hybrid immunoglobulin molecules can be produced (e.g., monomeric, homodimeric, heterodimeric, homotetrameric, heterotetrameric), depending upon the type of constant region selected and the portion used (e.g., light chain constant region, heavy chain constant region (such as $\gamma 1$, $\gamma 2$, $\gamma 3$, $\gamma 4$, $\alpha 1$, $\alpha 2$, δ , ϵ , and μ constant regions obtained from IgG, IgA, IgD, IgE, or IgM), and portions thereof) in the fusion polypeptide, and whether they are assembled into multimeric forms with each other and/or with other hybrid immunoglobulins or immunoglobulin chains (see Capon et al., U.S. Patent No. 5,428,130 and 5,225,538). In a preferred embodiment, the fusion protein comprises a complete heavy chain constant region or at least a functionally active hinge region, CH2 and CH3 domain. A particular constant region (e.g., IgG1), variant or portions thereof can be selected to tailor effector function. For example, a mutated constant region (variant) can be incorporated into a fusion protein to minimize binding to Fc receptors (Example 3; winter et al.,

GB 2,209,757 B; and Morrison et al., WO 89/07142), and/or to fix complement (WO 94/29351, December 22, 1994), etc.

[0038] Examples of "primate MAdCAM" proteins include proteins encoded by a human or macaque MAdCAM-1 nucleic acid of the present invention, such as a protein having an amino acid sequence as set forth or substantially as set forth in Figure 1 (SEQ ID NO:2), Figure 2 (SEQ ID NO:4) or Figure 3 (SEQ ID NO:6), and functional portions thereof. In a preferred embodiment, a primate MAdCAM or variant has an amino acid sequence which is at least about 55% similar, more preferably at least about 75% similar, and still more preferably at least about 90% similar, to a protein shown in Figure 1 (SEQ ID NO:2), Figure 2 (SEQ ID NO:4) or Figure 3 (SEQ ID NO:6).

MAdCAM structure

[0039] Murine MAdCAM-1, a member of the immunoglobulin supergene family, is a multi-domain molecule, comprising both immunoglobulin-related and mucin-like sequences (Briskin, M.J., et al., *Nature*, 363:461 (1993)). As indicated in Figure 6, the murine form contains two amino-terminal immunoglobulin-like domains are homologous to domains of the Ig-like adhesion receptors, ICAM-1 and VCAM-1, and are implicated in integrin binding. The third (membrane proximal) immunoglobulin-like domain, while unrelated to adhesion receptors of this class, shares homology with another mucosal-related immunoglobulin superfamily member, IgA. In addition to the three immunoglobulin-like domains, murine MAdCAM-1 has a serine/threonine-rich mucin-like domain between the second and third Ig-like domains. These structural elements suggest that MAdCAM-1 facilitates more than one function in cell adhesion cascades, and recent studies of murine MAdCAM-1 support a role for MAdCAM-1 in both selectin and integrin binding (Moore, K.L., et al., *J. Cell. Biol.*, 118:445 (1992); Bargatze, R.F., et al., *Immunity*, 3:99-108 (1995)). Also in this regard, it has been reported that murine MAdCAM-1, when expressed in mesenteric lymph nodes can present L-selectin binding carbohydrates associated with the peripheral node addressin epitope, MECA-79 (Berg, E.L., et al., *Nature*, 366:695 (1993)).

[0040] As described herein human and macaque MAdCAM-1 proteins have two immunoglobulin-like (Ig-like) domains which are homologous to the two amino-terminal immunoglobulin-like integrin binding domains of murine MAdCAM-1 (Figures 1-3, and 6). However, the similarity of sequences within the region homologous to the mucin/IgA domain of murine MAdCAM-1 is much less apparent. The membrane proximal regions of the human and macaque receptors exhibit considerable variation (as compared with each other or murine MAdCAM-1) with respect to the length of the mucin-like sequence and the lack of a membrane proximal Ig (IgA like) domain.

[0041] Two isoforms of human MAdCAM-1 have been identified which exhibited single amino acid polymorphisms and variation in the number of copies of a serine/threonine/proline rich repeat in the mucin region. These two isoforms appear to be encoded in genomic DNA, suggesting allelic variation and/or alternative processing of this sequence. These two isoforms may serve as alternative mechanisms of regulating $\alpha 4\beta 7$ binding affinity and/or presenting carbohydrates for selectin binding. The presence of these Ig-like and mucin domains in primate MAdCAMs described herein is also consistent with role in selectin as well as integrin binding.

[0042] Recent domain swapping experiments in murine MAdCAM-1 have shown that, although domain one of MAdCAM-1 can weakly bind $\alpha 4\beta 7$, adhesion is poor in the absence of strong integrin activation. The two amino-terminal Ig-like domains (which are similar to domains of ICAM-1 and VCAM-1) are sufficient for $\alpha 4\beta 7$ binding activity in an activation independent manner comparable to that of wild type murine MAdCAM-1.

[0043] A short motif (GLDTSL) present in domain one of murine MAdCAM-1, is conserved and required for integrin binding in other Ig-like adhesion receptors, including of domain one of ICAM-1, ICAM-2, and ICAM-3, and domains 1 and 4 of VCAM-1 (Staunton, D.E., *Cell*, 52: 925-33 (1988); Staunton, D.E., et al., *Nature*, 339:61 (1989); Osborn, L., et al., *Cell*, 59:1203 (1989); Fawcett, J., et al., *Nature*, 360:481 (1992)). This sequence, G-(I/L)-(D/E)-(T/S)-(P/S)-L, is located between β sheets c and d of these integrin binding domains. The GLDTSL motif was found in the primate MAdCAMs characterized here.

[0044] Mutagenesis of E34 (Glu³⁴) in this motif of domain 1 of ICAM-1 (underlined above) and of D40 (Asp⁴⁰) in VCAM-1 (in bold face above) had profound effects on binding of LFA-1 and $\alpha 4\beta 1$, respectively (Osborn, L., et al., *J. Cell. Biol.*, 124:601-608 (1994); Renz, M.E., et al., *J. Cell. Biol.*, 125:1395-1406 (1994); Staunton, D.E., et al., *Cell*, 61:243-254 (1990); Vonderheide, R.H., et al., *J. Cell. Biol.*, 125:215-222 (1994)). More recently, a fragment of VCAM-1 comprising the two N-terminal domains was subjected to crystallographic structure determination (Jones, E.Y., et al., *Nature*, 373: 539-544 (1995); Wang, J-H, et al., *Proc. Natl. Acad. Sci. USA.*, 92:5714-5718 (1995)). The conserved motif in VCAM-1 (QIDSPL) appears to be highly exposed on the N-terminal portion of the CD loop of the first Ig domain in a position that appears to be readily accessible to integrins.

[0045] A nucleotide substitution in this motif of murine MAdCAM-1, resulting in a change at amino acid 61 from leucine to arginine (L61-R61), abolishes MAdCAM-1 interactions with resting lymphocytes expressing $\alpha 4\beta 7$. Therefore, murine MAdCAM-1 also requires this conserved amino acid motif, GLDTSL, within the computer predicted CD loop of its N-terminal domain for binding its integrin ligand, $\alpha 4\beta 7$.

[0046] Comparisons of human MAdCAM cDNA clones 4 and 20 (Figures 1 and 2) revealed that the amino-terminal 225 amino acids are identical in clones 4 and 20. This region comprises a predicted 18 amino acid hydrophobic leader

or signal sequence, and two immunoglobulin-like domains. This region can be aligned with primate and murine MAdCAM-1, and displays the following conserved features: (1) a predicted signal peptide (identical in the human proteins, and similar to the macaque and murine signal peptides); (2) two pairs of cysteine residues in the first Ig-like domain, the cysteines of each pair being separated by 3 amino acids; (3) a sequence of nine amino acids (which contains the "LDTSL" motif) in the predicted C-D loop of Ig-like domain 1, and is implicated as a general integrin recognition site (identical in each primate clone); and (4) an uncharacteristically large second immunoglobulin-like domain. The size of the second Ig-like domain, with approximately 70 amino acids between cysteine residues would classify it as a "V" (variable) type domain, in contrast with the C2 type (constant) domains which are more typically found in the Ig-like adhesion receptors (Hunkapiller, T., et al., *Adv. in Immunol.*, 44:1-62 (1989); Williams, A.F., et al., *Annu. Rev. Immunol.*, 6:381-405 (1988)). Within this domain is an extended C'-E loop containing an abundance of negatively charged residues, which is common to each primate, murine and human MAdCAM-1 clone characterized, but which is not seen in related adhesion receptors.

[0047] The next region found in clones 4 and 20 is analogous to the mucin domain of murine MAdCAM-1, due to a prevalence of serine, threonine and proline (69% for clone 4 and 76% for clone 20) residues (boxed in Figure 1 and Figure 2). This region, although similar in amino acid composition to murine MAdCAM-1, is highly divergent from murine MAdCAM-1. Therefore, selection for conservation of the integrin binding Ig-like domains appears greater than that of the mucin sequences. The human MAdCAM-1 domain is 71 amino acids long in clone 4, and 47 amino acids long in clone 20. This region also contains two polymorphisms: (1) a polymorphism at amino acid 240, which is proline (P) in clone 4 and serine (S) in clone 20; and (2) a polymorphism at amino acid 242, which is asparagine (N) in clone 4 and aspartate (D) in clone 20. In addition, the human mucin domains contain a repeat of 8 amino acids consisting of the sequence PPDTS(Q/P)E, which appears eight times in clone 4 and five times in clone 20.

[0048] Since the human mucin domain is highly repetitive, truncation of three repeats in clone 20 relative to clone 4 could be the result of processes such as alternative splicing or mutation (e.g., an aberrant recombination event) that maintain the reading frame, yielding a receptor that is functional with respect to integrin binding, and suggesting that some or all of the mucin sequences are dispensable for integrin binding. Consistently, it has been shown that Ig-like domains 1 and 2 of murine MAdCAM-1 are sufficient for activation-independent adhesion to $\alpha 4\beta 7$, indicating that murine mucin sequences are dispensable for integrin binding. Also of interest in this regard, the macaque clone which was isolated lacks most of the repeat region.

[0049] The remaining C-terminal 110 amino acids are identical between clones 4 and 20: 47 amino acids precede a predicted hydrophobic transmembrane segment of 20 amino acids, which is followed by a cytoplasmic tail of 43 amino acids. The 47 amino acids immediately C-terminal to the mucin region are in a region corresponding to the IgA-like Ig domain of murine MAdCAM-1. Although the human and macaque proteins are similar in this region, they are divergent from murine MAdCAM-1. Compared with murine MAdCAM-1, the human proteins are 59 amino acids shorter in this region, and lack any characteristics of an Ig-like domain. The transmembrane domains of all the receptors are similar, but the cytoplasmic tail is considerably longer (43 amino acids) in human (26 in primate and 20 in the mouse) MAdCAM-1.

Method of Producing Recombinant Proteins

[0050] Another aspect of the invention relates to a method of producing a primate MAdCAM or variant (e.g., portion) thereof. Recombinant protein can be obtained, for example, by the expression of a recombinant DNA molecule encoding a primate MAdCAM or variant thereof in a suitable host cell, for example.

[0051] Constructs suitable for the expression of a primate MAdCAM or variant thereof are also provided. The constructs can be introduced into a suitable host cell, and cells which express a recombinant primate MAdCAM or variant thereof, can be produced and maintained in culture. Such cells are useful for a variety of purposes, and can be used in adhesion assays (e.g., in an assay to screen for ligands and/or candidate inhibitors of MAdCAM-mediated adhesion), in the production of protein for characterization, isolation and/or purification, (e.g., affinity purification), and as immunogens, for instance. Suitable host cells can be procaryotic, including bacterial cells such as *E. coli*, *B. subtilis* and or other suitable bacteria, or eucaryotic, such as fungal or yeast cells (e.g., *Pichia pastoris*, *Aspergillus species*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*), or other lower eucaryotic cells, and cells of higher eucaryotes such as those from insects (e.g., Sf9 insect cells) or mammals (e.g., Chinese hamster ovary cells (CHO), COS cells, HuT 78 cells, 293 cells). (See, e.g., Ausubel, F.M. et al., eds. *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons Inc., (1993)). In one embodiment, host cells capable of expressing membrane-bound mature protein are used. In another embodiment, host cells capable of secreting a soluble MAdCAM (e.g., soluble MAdCAM, such as MAdCAM lacking the C-terminal transmembrane region and cytoplasmic tail).

[0052] Host cells which produce a recombinant primate MAdCAM or variants thereof can be produced as follows. For example, a nucleic acid encoding all or part of the coding sequence for the desired protein can be inserted into a nucleic acid vector, e.g., a DNA vector, such as a plasmid, virus or other suitable replicon for expression. A variety of vectors are available, including vectors which are maintained in single copy or multiple copy, or which become integrated into the host cell chromosome.

[0053] The transcriptional and/or translational signals of a a MAdCAM-1 gene can be used to direct expression. Alternatively, suitable expression vectors for the expression of a nucleic acid encoding all or part of the coding sequence of the desired protein are available. Suitable expression vectors can contain a number of components, including, but not limited to one or more of the following: an origin of replication; a selectable marker gene; one or more expression control elements, such as a transcriptional control element (e.g., a promoter, an enhancer, terminator), and/or one or more translation signals; a signal sequence or leader sequence for membrane targeting or secretion (of primate origin or from a heterologous primate or non-primate species). In a construct, a signal sequence can be provided by the vector, the primate MAdCAM coding sequence, or other source.

[0054] A promoter is provided for expression in a suitable host cell. Promoters can be constitutive or inducible. In the vectors, the promoter is operably linked to a nucleic acid encoding the primate MAdCAM or variant thereof, and is capable of directing expression of the encoded polypeptide. A variety of suitable promoters for procaryotic (e.g.; lac, tac, T3, T7 promoters for *E. coli*) and eucaryotic (e.g., yeast alcohol dehydrogenase (ADH1), SV40, CMV) hosts are available.

[0055] In addition, the expression vectors typically comprise a selectable marker for selection of host cells carrying the vector, in the case of replicable expression vector, an origin or replication. Genes encoding products which confer antibiotic or drug resistance are common selectable markers and may be used in procaryotic (e.g., β -lactamase gene (ampicillin resistance), *Tet* gene for tetracycline resistance) and eucaryotic cells (e.g., neomycin (G418 or geneticin), gpt (mycophenolic acid), ampicillin, or hygromycin resistance genes). Dihydrofolate reductase marker genes permit selection with methotrexate in a variety of hosts. Genes encoding the gene product of auxotrophic markers of the host (e.g., *LEU2*, *URA3*, *HIS3*) are often used as selectable markers in yeast. Use of viral (e.g., baculovirus) or phage vectors, and vectors which are capable of integrating into the genome of the host cell, such as retroviral vectors, are also contemplated. The present invention also relates to cells carrying these expression vectors.

[0056] For example, a nucleic acid encoding a primate MAdCAM or variant thereof can be incorporated into the vector, operably linked to one or more expression control elements, and the construct can be introduced into host cells which are maintained under conditions suitable for expression, whereby the encoded polypeptide is produced. The construct can be introduced into cells by a method appropriate to the host cell selected (e.g., transformation, transfection, electroporation, infection). For production of a protein, host cells comprising the construct are maintained under conditions appropriate for expression, (e.g., in the presence of inducer, suitable media supplemented with appropriate salts, growth factors, antibiotic, nutritional supplements, etc.). The encoded protein (e.g., human MAdCAM-1) can be isolated from the host cells or medium.

[0057] Fusion proteins can also be produced in this manner. For example, some embodiments can be produced by the insertion of a primate MAdCAM cDNA or portion thereof into a suitable expression vector, such as Bluescript[®] II SK +/(Stratagene), pGEX-4T-2 (Pharmacia), pcDNA-3 (Invitrogen) and pET-15b (Novagen). The resulting construct is then introduced into a suitable host cell for expression. Upon expression, fusion protein can be isolated or purified from a cell lysate by means of a suitable affinity matrix (see e.g., Current Protocols in Molecular Biology (Ausubel, F.M. et al., eds., Vol. 2, Suppl. 26, pp. 16.4.1-16.7.8 (1991)). In addition, affinity labels provide a means of detecting a fusion protein. For example, the cell surface expression or presence in a particular cell fraction of a fusion protein comprising an antigen or epitope affinity label can be detected by means of an appropriate antibody.

Nucleic Acids, Constructs and Vectors

[0058] The present invention relates to isolated and/or recombinant (including, e.g., essentially pure) nucleic acids having sequences which encode a primate MAdCAM or variant thereof as described herein.

[0059] Nucleic acids referred to herein as "isolated" are nucleic acids separated away from the nucleic acids of the genomic DNA or cellular RNA of their source of origin (e.g., as it exists in cells or in a mixture of nucleic acids such as a library), and may have undergone further processing. "Isolated" nucleic acids include nucleic acids obtained by methods described herein, similar methods or other suitable methods, including essentially pure nucleic acids, nucleic acids produced by chemical synthesis, by combinations of biological and chemical methods, and recombinant nucleic acids which are isolated (see e.g., Daugherty, B.L. et al., Nucleic Acids Res., 19(9):2471-2476 (1991); Lewis, A.P. and J.S. Crowe, Gene, 101: 297-302 (1991)). Nucleic acids referred to herein as "recombinant" are nucleic acids which have been produced by recombinant DNA methodology, including those nucleic acids that are generated by procedures which rely upon a method of artificial recombination, such as the polymerase chain reaction (PCR) and/or cloning into a vector using restriction enzymes. "Recombinant" nucleic acids are also those that result from recombination events that occur through the natural mechanisms of cells, but are selected for after the introduction to the cells of nucleic acids designed to allow and make probable a desired recombination event.

[0060] In one embodiment, the nucleic acid or portion thereof encodes a protein or polypeptide having at least one property, activity or function characteristic of a primate MAdCAM (as defined herein), such as binding function (e.g., the ability to bind an $\alpha\beta 7$ integrin), and/or cellular adhesion molecule function (e.g., the ability to mediate cellular adhesion such as $\alpha\beta 7$ -dependent adhesion *in vitro* and/or *in vivo*), and/or an immunological property as defined herein.

[0061] The present invention also relates more specifically to isolated and/or recombinant nucleic acids or a portion thereof having sequences which encode human or macaque MAdCAM-1 or variants thereof.

[0062] The invention further relates to isolated and/or recombinant nucleic acids that are characterized by:

- (1) their ability to hybridize to (a) a nucleic acid encoding a primate MAdCAM, such as a nucleic acid having a nucleotide sequence as set forth or substantially as set forth in Figure 1 (SEQ ID NO:1), Figure 2 (SEQ ID NO:3), or Figure 3 (SEQ ID NO:5); (b) the complement of any one of (a); or (c) portions of either of the foregoing (e.g., a portion comprising the open reading frame); or
- (2) by their ability to encode a polypeptide having the amino acid sequence of a primate MAdCAM (e.g., SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6); or
- (3) by both characteristics.

[0063] In one embodiment, the nucleic acid shares at least about 50% nucleotide sequence similarity to any one of the nucleotide sequences shown in Figure 1, Figure 2, or Figure 3 (SEQ ID NO:1, 3, or 5, respectively) or to one of the MAdCAM coding regions thereof. More preferably, the nucleic acid shares at least about 75% nucleotide sequence similarity, and still more preferably, at least about 90% nucleotide sequence similarity, to any one of the sequences shown in Figure 1, Figure 2, or Figure 3 (SEQ ID NO:1, 3, or 5, respectively) or to one of the MAdCAM coding regions thereof.

[0064] Isolated and/or recombinant nucleic acids meeting these criteria comprise nucleic acids having sequences identical to sequences of naturally occurring primate MAdCAMs or variants of the naturally occurring sequences. Such variants include mutants differing by the addition, deletion or substitution of one or more residues, modified nucleic acids in which one or more residues are modified (e.g., DNA or RNA analogs), and mutants comprising one or more modified residues.

[0065] Nucleic acids of the present invention, including those which hybridize to a selected nucleic acid as described above, can be detected or isolated under high stringency conditions or moderate stringency conditions, for example. "High stringency conditions" and "moderate stringency conditions" for nucleic acid hybridizations are explained at pages 2.10.1-2.10.16 (see particularly 2.10.8-11) and pages 6.3.1-6 in Current Protocols in Molecular Biology (Ausubel, F.M. et al., eds., Vol. 1, Suppl. 26, 1991), the teachings of which are hereby incorporated by reference. Factors such as probe length, base composition, percent mismatch between the hybridizing sequences, temperature and ionic strength influence the stability of nucleic acid hybrids. Thus, high or moderate stringency conditions can be determined empirically, and depend in part upon the characteristics of the known nucleic acid (e.g., DNA) and the other nucleic acids to be assessed for hybridization thereto.

[0066] Isolated and/or recombinant nucleic acids that are characterized by their ability to hybridize (e.g. under high or moderate stringency conditions) to (a) a nucleic acid encoding a primate MAdCAM (for example, those nucleic acids depicted in Figure 1 (SEQ ID NO:1), Figure 2 (SEQ ID NO:3), and Figure 3 (SEQ ID NO:5), (b) the complement of such nucleic acids, (c) or a portion thereof, can also encode a protein or polypeptide having at least one property, activity or function characteristic of a primate MAdCAM (as defined herein), such as binding function (e.g., the ability to bind an $\alpha 4\beta 7$ integrin), and/or cellular adhesion molecule function (e.g., the ability to mediate cellular adhesion such as $\alpha 4\beta 7$ -dependent adhesion *in vitro* and/or *in vivo*), and/or an immunological property as defined herein. Preferred nucleic acids have lengths of at least about 40 nucleotides, more preferably at least about 50, and still more preferably at least about 75 nucleotides.

[0067] The binding function of a primate MAdCAM or variant thereof which is encoded by a nucleic acid of the present invention can be detected by standard assays for ligand binding (e.g., assays which monitor formation of a complex between isolated and/or recombinant MAdCAM and an $\alpha 4\beta 7$ integrin) or standard adhesion assays (e.g., in which adhesion between a first cell expressing a recombinant primate MAdCAM, and a second cell bearing an $\alpha 4\beta 7$ integrin is monitored), or other suitable methods. Binding and/or adhesion assays or other suitable methods can also be used in procedures for the identification and/or isolation of nucleic acids which encode a polypeptide of the present invention (see e.g., Example 1). The antigenic properties of proteins or polypeptides encoded by nucleic acids of the present invention can be determined by immunological methods employing antibodies that bind to a primate MAdCAM, such as immunoblotting, immunoprecipitation and immunoassay (e.g., radioimmunoassay, ELISA).

[0068] Nucleic acids of the present invention can be used in the production of proteins or polypeptides. For example, a nucleic acid (e.g., DNA) encoding a primate MAdCAM can be incorporated into various constructs and vectors created for further manipulation of sequences or for production of the encoded polypeptide in suitable host cells as described above.

[0069] A further embodiment of the invention is antisense nucleic acid, which is complementary, in whole or in part, to a target molecule comprising a sense strand, and can hybridize with the target molecule. The target can be DNA, or its RNA counterpart (i.e., wherein T residues of the DNA are U residues in the RNA counterpart). When introduced into a cell, antisense nucleic acid can inhibit the expression of the gene encoded by the sense strand. Antisense nucleic

acids can be produced by standard techniques.

[0070] In a particular embodiment, the antisense nucleic acid is wholly or partially complementary to and can hybridize with a target nucleic acid, wherein the target nucleic acid can hybridize to a nucleic acid having the sequence of the complement of the top strand shown in Figure 1 (SEQ ID NO:1), Figure 2 (SEQ ID NO:3), or Figure 3 (SEQ ID NO:5). For example, antisense nucleic acid can be complementary to a target nucleic acid having the sequence shown as the top strand of the open reading frame in Figure 1 (SEQ ID NO:1), Figure 2 (SEQ ID NO:3), or Figure 3 (SEQ ID NO:5), or to a portion thereof sufficient to allow hybridization. In another embodiment, the antisense nucleic acid is wholly or partially complementary to and can hybridize with a target nucleic acid which encodes a primate MAdCAM.

[0071] The nucleic acids can also be used as probes (e.g., in *in situ* hybridization) to assess associations between inflammatory bowel disease (IBD) (or other conditions) and increased expression of primate MAdCAM in affected tissues. The nucleic acids can also be used as probes to detect and/or isolate (e.g., by hybridization with RNA or DNA) polymorphic or allelic variants, for example, in a sample (e.g., inflamed tissue) obtained from a primate. Moreover, the presence or frequency of a particular variant in a sample(s) obtained from one or more affected primates, as compared with a sample(s) from normal primate(s), can be indicative of an association between inflammatory bowel disease (IBD) (or other conditions) and a particular variant, which in turn can be used in the diagnosis of the condition.

[0072] As described in the Examples, a cDNA clone encoding macaque MAdCAM-1 was isolated by expression cloning, and the cDNA was used as a probe to screen a human cDNA library. Two distinct nucleic acids encoding human MAdCAM-1 were isolated and characterized. Additional human, macaque or other primate genes or cDNAs can be obtained. For example, the genes described here, or sufficient portions thereof, whether isolated and/or recombinant or synthetic, can be used as probes or primers to detect and/or recover additional nucleic acids encoding primate MAdCAMs or variants thereof from a suitable source such as a primate genomic or cDNA library, according to methods described herein or other suitable methods (e.g., by hybridization, PCR, expression cloning or other suitable techniques).

[0073] In one embodiment, nucleic acids encoding primate MAdCAM are producible by methods such as PCR amplification. For example, appropriate primers (e.g., a pair of primers or nested primers) can be designed which comprise a sequence which is complementary or substantially complementary to a portion of a primate MAdCAM cDNA described herein. For instance, primers complementary to the 5'- or 3'-ends of the coding sequence and/or flanking the coding sequence can be designed. Such primers can be used in a polymerase chain reaction with a suitable template nucleic acid to obtain nucleic acid encoding primate MAdCAM, for example. Suitable templates include e.g., constructs described herein (such as pcD3PMAAd, pcD3HuMAd-4 or pcD3HuMAd-20), a cDNA library or another suitable source of primate (e.g., human) cDNA or genomic DNA. Primers can contain portions complementary to flanking sequences of the construct selected as template as appropriate.

[0074] Additional genes or cDNAs can be used to express primate MAdCAM, with utilities corresponding to those described herein, and can be used in the production of constructs, host cells, and antibodies using methods described herein. The approaches described herein, including, but not limited to, the approaches used to isolate and manipulate macaque and human MAdCAM-1, to construct vectors and host strains, and to produce and use the proteins, to produce antibodies, etc., can be applied to other primates.

Therapeutic Methods and Compositions

[0075] The present invention also provides antibodies which (1) can bind a "primate MAdCAM" *in vitro* and/or *in vivo*; and/or (2) can inhibit an activity or function characteristic of a "primate MAdCAM", such as binding function (e.g., the ability to bind an $\alpha 4\beta 7$ integrin) and/or cellular adhesion molecule function (e.g., the ability to mediate cellular adhesion such as $\alpha 4\beta 7$ -dependent adhesion *in vitro* and/or *in vivo*). Such antibodies include antibodies which can bind a human or macaque MAdCAM encoded by cDNA clone 4, cDNA clone 20 or cDNA clone 31D. Also encompassed are antibodies which can bind a naturally occurring or endogenous primate MAdCAM (e.g., human MAdCAM). Preferably the antibodies are capable of selective binding of primate MAdCAM *in vitro* and/or *in vivo* (e.g., bind selectively to primate MAdCAM expressed in mucosal tissue and/or spleen (e.g., as assessed immunohistologically)).

[0076] In one embodiment, the antibodies can bind primate MAdCAM and inhibit binding of "primate MAdCAM" to an $\alpha 4\beta 7$ integrin (e.g., human), thereby inhibiting cellular adhesion mediated by MAdCAM, preferably selectively. Such an antibody can inhibit $\alpha 4\beta 7$ -dependent cellular adhesion to cells bearing an $\alpha 4\beta 7$ integrin, such as leukocytes (especially lymphocytes such as T or B cells) *in vitro* and/or *in vivo*. For example, eleven hybridomas were identified which produced antibodies which specifically inhibit the adhesion of RPMI 8866 cells to MAdCAM-1 (Example 2, hybridomas designated 10G4, 8C1, 10G3, 9G12, 9E4, 7H12, 10F2, 10A6, 1E5, 2F5, 7G11). Thus, antibodies which can inhibit cellular adhesion of cells bearing an $\alpha 4\beta 7$ integrin to vascular endothelial cells in mucosal tissues, including gut-associated tissues or lymphoid organs are encompassed by the antibodies of the present invention.

[0077] Preferably, the antibodies can bind a primate MAdCAM with high affinity (for example, a K_a in the range of about 1 - 10 nM, or a K_d in the range of about 1×10^{-8} to 1×10^{-10} mol⁻¹).

[0078] The antibodies of the present invention are useful in a variety of applications, including processes, research,

diagnostic and therapeutic applications. For instance, they can be used to isolate and/or purify primate MAdCAM or variants thereof (e.g., by affinity purification or other suitable methods), and to study MAdCAM structure (e.g., conformation) and function.

[0079] The antibodies of the present invention can also be used to modulate MAdCAM function in diagnostic (e.g., in vitro) or therapeutic applications. For instance, antibodies can act as inhibitors of to inhibit (reduce or prevent) binding function and/or cellular adhesion molecule function of a primate MAdCAM as described herein.

[0080] In addition, antibodies of the present invention can be used to detect and/or measure the level of a primate MAdCAM in a sample (e.g., tissues or body fluids, such as an inflammatory exudate, blood, serum, bowel fluid, or on cells transfected with a nucleic acid of the present invention). For example, a sample (e.g., tissue and/or fluid) can be obtained from a primate and a suitable immunological method can be used to detect and/or measure primate MAdCAM levels, including methods such as enzyme-linked immunosorbent assays (ELISA), including chemiluminescence assays, radioimmunoassay, and immunohistology. In one embodiment, a method of detecting a selected primate MAdCAM in a sample is provided, comprising contacting a sample with an antibody which binds an isolated primate MAdCAM under conditions suitable for specific binding of said antibody to the selected primate MAdCAM, and detecting antibody-MAdCAM complexes which are formed.

[0081] In an application of the method, antibodies reactive with a primate MAdCAM-1 can be used to analyze normal versus inflamed tissues in human and non-human primates for primate MAdCAM reactivity and/or expression (e.g., immunohistologically). Thus, the antibodies of the present invention permit immunological methods of assessment of expression of primate (e.g., human MAdCAM-1) in normal versus inflamed tissues, through which the presence of disease, disease progress and/or the efficacy of anti-primate MAdCAM-1 therapy in inflammatory disease can be assessed.

[0082] The present invention also provides "primate MAdCAM" as defined herein, including functional variants, such as soluble primate MAdCAM (e.g., lacking the all or part of the transmembrane region and cytoplasmic tail, such that the protein secreted) and functional fusion proteins (e.g., hybrid immunoglobulins comprising a primate MAdCAM moiety fused at its C-terminus, to the N-terminus of an immunoglobulin moiety). These molecules are useful in a variety of applications, including processes, research, diagnostic and therapeutic applications.

[0083] For example, primate MAdCAM, MAdCAM-Ig fusion proteins or other recombinant soluble primate MAdCAM molecules can be used in assays to identify ligands or inhibitors (e.g., a blocking antibody) of primate MAdCAM: α 4 β 7 interaction. As used herein, an inhibitor is a compound which inhibits (reduces or prevents) the binding of primate MAdCAM-1 to a ligand, including α 4 β 7 integrin, and/or which inhibits the triggering of a cellular response mediated by the ligand. An effective amount is an amount sufficient to achieve inhibition of binding or adhesion to primate MAdCAM-1 and/or signalling (e.g., an amount sufficient to inhibit adhesion of a cell bearing a primate MAdCAM-1 ligand (including α 4 β 7 integrins, such as human α 4 β 7 integrin, and its primate homologs)) to isolated and/or recombinant primate MAdCAM.

[0084] In one aspect, a method of detecting or identifying a ligand of primate MAdCAM or an agent which binds a primate MAdCAM is provided, in which an (i.e., one or more) agent to be tested (or a candidate ligand) is contacted with an isolated and/or recombinant "primate MAdCAM", including "functional variants", as defined herein under conditions suitable for binding of ligand thereto, and the formation of a complex between said agent and primate MAdCAM is detected. In one embodiment, an agent to be tested is combined with a host cell expressing recombinant primate MAdCAM or a functional variant under conditions suitable for binding of ligand thereto. In one embodiment, the primate MAdCAM or functional variant is labeled with a suitable label (e.g., fluorescent label, isotope label), and binding is determined by detection of the label. Specificity of binding can be assessed by competition or displacement, for example, using unlabeled agent, an unlabeled isolated and/or recombinant primate MAdCAM or functional variant, or a second ligand of primate MAdCAM as competitor.

[0085] In another aspect, a method of detecting an inhibitor of cellular adhesion mediated by primate MAdCAM is provided. In one embodiment, an agent to be tested is combined with a ligand of primate MAdCAM, and an isolated and/or recombinant primate MAdCAM or functional variant (e.g., fusion protein) under conditions suitable for binding of ligand thereto. The formation of a complex between the ligand and primate MAdCAM or the functional variant is monitored. A decrease in binding of ligand in the presence of the agent relative to a suitable control (e.g., binding in the absence of agent) is indicative that the agent is an inhibitor. For example, the fusion proteins and assays described in Example 3 can be used to detect inhibitors. An agent to be tested can also be combined with a first cell expressing a recombinant primate MAdCAM, and a second cell bearing an α 4 β 7 integrin under conditions suitable for adhesion of said first cell to said second cell. Adhesion between said first and second cells can be monitored, and decreased adhesion (reduced or abolished) as compared with a suitable control is indicative that the agent is an inhibitor. A cell or cells which naturally express a ligand for MAdCAM-1, such as a leukocyte (e.g., an α 4 β 7⁺ B lymphocyte, T lymphocyte) or other cell which expresses a ligand for MAdCAM-1 (e.g., a recombinant cell) can be used.

[0086] Assays such as those described in Example 3 can be used to identify compounds which inhibit binding *in vitro*. As shown herein, fusion proteins comprising a primate MAdCAM moiety (two chimeric MAdCAM-Ig fusions) can bind

to $\alpha 4\beta 7$ positive lymphocytes in solution. Thus, primate MAdCAM, including functional variants, particularly soluble primate MAdCAM molecules and fusion proteins such as the chimeric MAdCAM-Ig fusions described in Example 3, provide candidate inhibitors of $\alpha 4\beta 7$:MAdCAM interaction and of *in vivo* lymphocyte recruitment to inflammatory sites, which can be useful in therapy as described hereinbelow. The *in vivo* efficacy of these molecules can be assessed using methods described herein (see e.g., Examples 4 and 5) or other suitable methods. For example, primate models such as those described in Example 5 can be used. The CD45RB^{Hi}/SCID model provides a mouse model with similarity to both Crohn's disease and ulcerative colitis (Example 4, Powrie, F. et al., *Immunity*, 1: 553-562 (1994)). Efficacy in this model can be assessed using an experimental protocol similar to the one used for monoclonal antibodies (Example 4). Parameters such as inhibition of recruitment of ¹¹¹In-labeled cells to the colon and reduction in the number of CD4⁺ T lymphocytes in the lamina propria of the large intestine after administration (e.g., intravenous (i.v.), intraperitoneally (i.p.) and per oral (p.o.)) can be assessed. Knockout mice which develop intestinal lesions similar to those of human inflammatory bowel disease have also been described (Strober, W. and Ehrhardt, R.O., *Cell*, 75: 203-205 (1993)), and NOD mice provide an animal model of insulin-dependent diabetes mellitus.

[0087] The invention further relates to the discovery that diseases associated with leukocyte recruitment to the gastrointestinal tract, such as IBD, or other mucosal tissues can be treated by inhibiting MAdCAM binding to the $\alpha 4\beta 7$ integrin and/or triggering of $\alpha 4\beta 7$ -mediated cellular responses. Compounds or agents which inhibit binding include "primate MAdCAM" as defined herein, including soluble primate MAdCAM molecules and fusion proteins, as well as antibodies or antigen binding fragments thereof which bind MAdCAM and/or the $\alpha 4\beta 7$ integrin. Antibodies which can be used in the method include recombinant or non-recombinant polyclonal, monoclonal, chimeric, humanized and/or anti-idiotypic antibodies.

[0088] Monoclonal antibodies that bind MAdCAM or $\alpha 4\beta 7$ have been described. For example, MECA-367 is an anti-MAdCAM antibody of the IgG2a subtype and is described in Gallatin et al., *Nature*, 304: 30 (1983) and Michie et al., *Am. J. Pathol.*, 143: 1688-1698 (1993). ACT-1 is a monoclonal antibody which binds the $\alpha 4\beta 7$ integrin (Lazarovits et al., *J. Immunol.*, 133: 1857 (1984); Schweighoffer et al., *J. Immunol.*, 151: 717-729 (1993)). FIB 21 binds the $\beta 7$ chain is described and characterized in Berlin et al., *Cell*, 74: 184-195 (1993); Andrew, D.P. et al., *J. Immunol.*, 153: 3847-3861 (1994)).

[0089] Other polyclonal or monoclonal antibodies, such as antibodies which bind to the same or similar epitopes as the antibodies described above, can be made according to methods described herein, methods known in the art or other suitable methods (such as Kohler et al., *Nature*, 256:495-497 (1975), Harlow et al., 1988, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor, NY) or *Current Protocols in Molecular Biology*, Vol. 2 (Supplement 27, Summer '94), Ausubel et al., Eds. (John Wiley & Sons: New York, NY), Chapter 11 (1991)). Antibodies can also be produced which can compete with any one of the antibodies produced by the hybridoma cell lines designated 10G4, 8C1, 10G3, 9G12, 9E4, 7H12, 10F2, 10A6, 1E5, 2F5, or 7G11 for binding to a cell bearing an $\alpha 4\beta 7$ integrin, preferably human $\alpha 4\beta 7$ integrin.

[0090] For example, antibodies can be raised against an appropriate immunogen in a suitable mammal (e.g., a mouse, rat, rabbit or sheep). Immunogens include, for example, MAdCAM, $\alpha 4\beta 7$, or immunogenic fragments thereof. For example, a primate MAdCAM or a variant thereof can be produced and used as an immunogen to raise antibodies in a suitable immunization protocol.

[0091] Antibody-producing cells (e.g., a lymphocyte) can be isolated from, for example, the lymph nodes or spleen of an immunized animal. The cells can then be fused to a suitable immortalized cell (e.g., a myeloma cell line), thereby forming a hybridoma. Fused cells can be isolated employing selective culturing techniques. Cells which produce antibodies with the desired specificity can be selected by a suitable assay (e.g., ELISA) (see e.g., Example 2).

[0092] In one embodiment, the immunogen can be an antibody which binds, for example, MAdCAM, $\alpha 4\beta 7$, or immunogenic fragments thereof. The antibody raised thereby can be an anti-idiotypic antibody, which can also be used in the present invention (U.S. Patent No. 4,699,880).

[0093] Single chain antibodies, and chimeric, humanized or primatized (CDR-grafted or resurfaced, such as, according to EP 0,592,406; Padlan *et al.*, April 13, 1994) antibodies, as well as chimeric or CDR-grafted single chain antibodies, comprising portions derived from different species, can also be used in the invention. The various portions of these antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or humanized chain can be expressed to produce a contiguous protein. See, e.g., Cabilly et al., U.S. Patent No. 4,816,567; Cabilly et al., European Patent No. 0,125,023 B1; Boss et al., U.S. Patent No. 4,816,397; Boss et al., European Patent No. 0,120,694 B1; Neuberger, M.S. et al., WO 86/01533; Neuberger, M.S. et al., European Patent No. 0,194,276 B1; Winter, U.S. Patent No. 5,225,539; and Winter, European Patent No. 0,239,400 B1. See also, Newman, R. et al., *BioTechnology*, 10: 1455-1460 (1992), regarding primatized antibody, and Ladner *et al.*, U.S. Patent No. 4,946,778 and Bird, R.E. et al., *Science*, 242:423-426 (1988)) regarding single chain antibodies.

[0094] In addition, functional fragments of antibodies, including fragments of chimeric, humanized, primatized or single chain antibodies, can also be produced. Functional fragments of the foregoing antibodies retain at least one binding function of the full-length antibody from which they are derived and, preferably, retain the ability to inhibit interaction.

For example, antibody fragments capable of binding to the $\alpha 4\beta 7$ integrin, MAdCAM or portion thereof include, but are not limited to, Fv, Fab, Fab' and F(ab')₂ fragments. Such fragments can be produced by enzymatic cleavage or by recombinant techniques. For instance, papain or pepsin cleavage can generate Fab or F(ab')₂ fragments, respectively. Alternatively, antibodies can be produced in a variety of truncated forms using antibody genes in which one or more stop codons has been introduced upstream of the natural stop site. For example, a chimeric gene encoding a F(ab')₂ heavy chain portion can be designed to include DNA sequences encoding the CH₁ domain and hinge region of the heavy chain.

[0095] Antibodies and antigen binding fragments thereof which can be used in the claimed method include antibodies which bind to MAdCAM and/or $\alpha 4\beta 7$, such as anti- $\beta 7$ chain antibodies. For example, antibodies from the group including FIB 21, FIB 30, FIB 504 and ACT-1 and mixtures thereof can be administered. Alternatively or in addition, antigen fragments of these antibodies can be administered.

[0096] Compounds or agents which inhibit the binding of MAdCAM and the $\alpha 4\beta 7$ integrin can be administered according to the claimed method in the treatment of diseases associated with leukocyte (e.g., lymphocyte, monocyte) infiltration of tissues (including recruitment and/or accumulation of leukocytes in tissues) which express the molecule MAdCAM-1. An effective amount of a compound or agent (i.e., one or more) is administered to an individual (e.g., a mammal, such as a human or other primate) in order to treat such a disease. For example, inflammatory diseases, including diseases which are associated with leukocyte infiltration of the gastrointestinal tract (including gut-associated endothelium), other mucosal tissues, or tissues expressing the molecule MAdCAM-1 (e.g., gut-associated tissues, such as venules of the lamina propria of the small and large intestine; and mammary gland (e.g., lactating mammary gland)), can be treated according to the present method. Similarly, an individual having a disease associated with leukocyte infiltration of tissues as a result of binding of leukocytes to cells (e.g., endothelial cells) expressing the molecule MAdCAM-1 can be treated.

[0097] Diseases which can be treated accordingly include inflammatory bowel disease (IBD), such as ulcerative colitis, Crohn's disease, ileitis, Celiac disease, nontropical Sprue, enteropathy associated with seronegative arthropathies, microscopic or collagenous colitis, eosinophilic gastroenteritis, or pouchitis resulting after proctocolectomy, and ileoanal anastomosis.

[0098] Pancreatitis and insulin-dependent diabetes mellitus are other diseases which can be treated using the present method. It has been reported that MAdCAM-1 is expressed by some vessels in the exocrine pancreas from NOD (nonobese diabetic) mice, as well as from BALB/c and SJL mice. Expression of MAdCAM-1 was reportedly induced on endothelium in inflamed islets of the pancreas of the NOD mouse, and MAdCAM-1 was the predominant addressin expressed by NOD islet endothelium at early stages of insulinitis (Hanninen, A., et al., J. Clin. Invest., 92: 2509-2515 (1993)). Further, accumulation of lymphocytes expressing $\alpha 4\beta 7$ within islets was observed, and MAdCAM-1 was implicated in the binding of lymphoma cells via $\alpha 4\beta 7$ to vessels from inflamed islets (Hanninen, A., et al., J. Clin. Invest., 92: 2509-2515 (1993)).

[0099] Examples of inflammatory diseases associated with mucosal tissues which can be treated according to the present method include mastitis (mammary gland), cholecystitis, cholangitis or pericholangitis (bile duct and surrounding tissue of the liver), chronic bronchitis, chronic sinusitis, asthma, and graft versus host disease (e.g., in the gastrointestinal tract). As seen in Crohn's disease, inflammation often extends beyond the mucosal surface, accordingly chronic inflammatory diseases of the lung which result in interstitial fibrosis, such as hypersensitivity pneumonitis, collagen diseases, sarcoidosis, and other idiopathic conditions can be amenable to treatment.

[0100] The compound is administered in an effective amount which inhibits binding of MAdCAM to the $\alpha 4\beta 7$ integrin. For therapy, an effective amount will be sufficient to achieve the desired therapeutic and/or prophylactic effect (such as an amount sufficient to reduce or prevent MAdCAM-mediated binding and/or signalling, thereby inhibiting leukocyte adhesion and infiltration and/or associated cellular responses). The compounds can be administered in a single dose or multiple doses. The dosage can be determined by methods known in the art and is dependent, for example, upon the individual's age, sensitivity, tolerance and overall well-being. Suitable dosages for antibodies can be from 0.1-1.0 mg/kg body weight per treatment.

[0101] According to the method, a compound or agent can be administered to an individual (e.g., a human) alone or in conjunction with another agent. A compound or agent can be administered before, along with or subsequent to administration of the additional agent. In one embodiment, more than one monoclonal antibody which inhibits the binding of leukocytes to endothelial MAdCAM is administered. Alternatively, a monoclonal antibody which inhibits the binding of leukocytes to endothelial ligands is administered in addition to an anti-MAdCAM or anti- $\beta 7$ antibody. For example, an antibody that inhibits the binding of leukocytes to an endothelial ligand other than MAdCAM, such as an anti-ICAM-1 or anti-VCAM-1 antibody can also be administered. In another embodiment, an additional pharmacologically active ingredient (e.g., sulfasalazine, an antiinflammatory compound, or a steroidal or other non-steroidal antiinflammatory compound) can be administered in conjunction with the compound or agent (e.g., the antibody of the present invention).

[0102] A variety of routes of administration are possible including, but not necessarily limited to parenteral (e.g., intravenous, intraarterial, intramuscular, subcutaneous injection), oral (e.g., dietary), topical, inhalation (e.g., intrabronchial, intranasal or oral inhalation, intranasal drops), or rectal, depending on the disease or condition to be treated.

Parenteral administration is a preferred mode of administration.

[0103] Formulation of a compound to be administered will vary according to the route of administration selected (e.g., solution, emulsion, capsule). An appropriate composition comprising the compound to be administered can be prepared in a physiologically acceptable vehicle or carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, Remington's Pharmaceutical Science, 16th Edition, Mack, Ed. 1980). For inhalation, the compound can be solubilized and loaded into a suitable dispenser for administration (e.g., an atomizer, nebulizer or pressurized aerosol dispenser).

EXEMPLIFICATION

[0104] The present invention will now be illustrated by the following Examples, which are not intended to be limiting in any way.

Introduction

[0105] Hybridization studies using zoo blots with murine MAdCAM DNA probes under low stringency conditions indicated that nucleotide conservation between murine MAdCAM-1 and higher species was poor. A functional expression approach was used to clone primate and human homologs, whereby cells transfected with cDNAs which conferred the ability to adhere to a target lymphocyte cell line expressing high levels of the MAdCAM-1 ligand ($\alpha 4\beta 7$) were identified and the cDNAs recovered. As human tissue sources were scarce, a primate homolog of MAdCAM-1 was first identified.

[0106] For expression cloning, a primate cDNA expression library, derived from mesenteric lymph nodes of a macaque, was made in a eukaryotic expression vector pRSVSPORT (from Gibco/BRL). A high efficiency transfection system using the CHO/P cell line (Heffernan, M. and J.D. Dennis, Nucleic Acids Res., 19: 85-92 (1991)) was used. The library was separated and individual pools (representing approximately 1,500 clones) were transfected in wells of 24 well tissue culture plates. Cell adhesion assays were performed to identify cDNAs which conferred an adhesive phenotype on T and B cell lines expressing the $\alpha 4\beta 7$ integrin, a known ligand for MAdCAM-1. Adhesion was identified microscopically by rosetting of the T and B cell lines on the transfected cells. A pool conferring the desired phenotype was subfractionated until a single full-length cDNA clone designated clone 31D was identified. DNA sequencing of the amino-terminal portion of the cDNA revealed homology of the macaque clone to murine MAdCAM-1 (Briskin, M.J., et al, Nature (Lond.), 363: 461-464 (1993)) at both the protein and nucleic acid level.

[0107] When introduced into CHO/P cells by transient transfection, the cDNA insert obtained from clone 31D directed the expression of a protein which could mediate binding to two cell lines which express $\alpha 4\beta 7$: (1) TK1, a murine T cell lymphoma (Butcher, E.C., et al., Eur. J. of Immunol., 10: 556-561 (1980)); and (2) RPMI 8866, a human B cell lymphoma (Erle, D.J., et al., J. Immunol., 153: 517-528 (1994)). Binding of TK1 cells to cells transfected with the macaque cDNA could be blocked by antibodies to either the $\alpha 4$ (MAb PS/2) or the $\beta 7$ (MAb FIB 504) integrins, and binding of RPMI 8866 to CHO/P cells transiently transfected with macaque cDNA (clone 31D in pSV-SPORT) was blocked by the anti- $\alpha 4\beta 7$ MAb, ACT-1. In control experiments, a cDNA encoding human VCAM-1 failed to bind the RPMI 8866 human B cell line. Jurkat cells, a T cell line which expresses $\alpha 4\beta 1$ and not $\alpha 4\beta 7$, was shown to bind VCAM-1, but failed to bind transfectants expressing macaque cDNA.

[0108] The cDNA encoding a primate (macaque) homolog of murine MAdCAM-1 was used as a probe to obtain a clone encoding a human homolog by hybridization. To obtain a human MAdCAM-1 clone, two cDNA libraries, one derived from histologically normal human mesenteric lymph node (MLN) and one derived from an inflamed MLN lymph node from a patient with Crohn's disease, were constructed in the λ Ziplox phage vector from Gibco/BRL. cDNA from the macaque clone was used to screen these libraries. Two different human cDNA clones of similar size were isolated. These clones each appeared to be full-length by preliminary sequence analysis. Analysis of human, as well as macaque, MAdCAM-1 cDNAs indicates that each of the encoded proteins has a predicted hydrophobic leader sequence (underlined in Figures 1-3), with the remaining portions of the proteins corresponding to predicted mature human or macaque MAdCAM-1, respectively.

[0109] To assess function, the human cDNA inserts were subcloned into the pCDNA3 expression vector (Invitrogen) and transient expression assays were used to demonstrate function. The human cDNAs can be expressed as functional proteins, and are capable of mediating specific binding to cells expressing $\alpha 4\beta 7$. Accordingly, these two human cDNA clones are designated as human MAdCAM-1 cDNAs.

[0110] Stable transfectants of both the primate and human cDNAs were generated in a mouse pre-B cell line, L1-2 and CHO cells. L1-2 transfectants were used to immunize mice and generate monoclonal antibodies against human MAdCAM-1. Antibodies capable of inhibiting the interaction between MAdCAM-1 and $\alpha 4\beta 7$ were identified. The produc-

tion of blocking antibodies directed against human MAdCAM-1 is a significant advance, as previous attempts to produce such blocking antibodies having cross-reactivity with the human homolog using murine MAdCAM-1 have failed.

Example 1. Cloning of Macaque and Human MAdCAM-1 cDNAs

RNA isolation and selection of message

[0111] Total RNA was isolated from (a) primate (macaque) mesenteric lymph nodes (MLN); (b) histologically normal human mesenteric lymph nodes; (c) human mesenteric lymph nodes (inflamed ileal nodes) from a patient with Crohn's disease; and (d) tissue culture cells by use of the CsTFA™ (cesium trifluoroacetate) reagent (Pharmacia; Cat. #17-087-02). Total RNA from mesenteric lymph node was obtained from two species of macaque (*Macaca fascicularis*, and *Macaca mulatta*), and was combined prior to isolation of poly-A RNA. Tissue was first snap frozen in liquid nitrogen and subjected to dounce homogenization in a solution consisting of 5.5 M guanidinium isothiocyanate, 25 mM sodium citrate, 0.5% sodium laurel sarcosine and 0.2 M 2-mercaptoethanol, while tissue culture cells ($1-5 \times 10^8$) were washed once in phosphate buffered saline (PBS) and homogenized by pipetting. A clarified lysate was then layered on a cushion of CsTFA and total RNA was pelleted by centrifugation for 20 hours at 30,000 RPM.

[0112] mRNA was selected by the polyAtract mRNA isolation system from Promega. The system uses a biotinylated oligo(dT) primer to hybridize (in solution) to poly A tails of eukaryotic messages. The hybrids were captured and washed at high stringency using streptavidin coupled to paramagnetic particles and a magnetic separation stand. mRNA was selected by a single purification in this system and the yields ranged from 1-2% of the total RNA yield. The integrity of both the total and mRNA was analyzed by gel electrophoresis and ethidium bromide staining.

cDNA synthesis

[0113] cDNA was synthesized using the Superscript™ lambda system (Cat. #18256-016) in conjunction with either the λZiplox™ vector (Gibco/BRL, Gaithersburg, MD, Cat. #19643-014) in the case of the human libraries, or the pSV-SPORT-1 vector (Gibco/BRL, Cat. #15388-010) in the case of the macaque library. The following modifications from the standard protocol were made. cDNA was labeled only in the first or second strand (but not both) with $\alpha^{32}\text{P}$ -dCTP and estimates of quantity were made by inspection of ethidium bromide staining of aliquots of cDNA fractions.

DNA Sequencing

[0114] The entire macaque and human MAdCAM-1 cDNAs were first isolated in the library vectors pSV-SPORT-1 and pZL1 (rescued from λZiplox™), respectively. Based on restriction mapping, fragments were subcloned into Bluescript® vectors (Stratagene) to facilitate sequencing from internal regions of the cDNAs. After sequence analysis of these clones, oligonucleotide primers were made to complete the sequence. Overlapping sequence of both strands was obtained. Sequence analysis utilized the sequenase™ 7-deaza-dGTP DNA sequencing kit with sequenase version 2.0 T7 DNA polymerase (United States Biochemical) and ^{35}S -dCTP (Amersham Life Science and New England Nuclear). The delta TAQ sequencing kit (USB) and gamma ^{32}P -ATP (Amersham) G-C rich sequence were also used for G-C rich sequences.

[0115] Sequences were entered and analyzed using the *Lasergene* system (DNASTAR, Inc.). Nucleotide sequence alignments were performed by the Clustal method with Weighted residue weight table, using a gap penalty of 10 and a gap length penalty of 10, and default parameters (Pairwise alignment parameters were: ktuple = 2, gap penalty = 5, window = 4, and diagonals saved = 4).

[0116] Amino acid sequence alignments were performed by the Clustal method with the PAM250 residue weight table, using a gap penalty of 10 and a gap length penalty of 10 and default parameters (Pairwise alignment parameters were: ktuple = 1, gap penalty = 3, window = 4, and diagonals saved = 5).

Preparation of macaque expression library

[0117] The size fractionation procedure was also modified slightly for construction of the macaque expression library to ensure large (>1.5 kb) inserts. After one round of fractionation, only the first (largest) fraction of cDNA was saved and the remaining fractions were pooled and subjected to a subsequent round of fractionation. The top fraction from the next round was pooled with the top fraction from the previous round and the second fraction from this round was also used. These two fractions were precipitated and put into ligations with the pSV-SPORT-1 vector and a fraction of each ligation was transformed into electrocompetent DH10B bacteria (Gibco) to estimate both the titer of the library and the average insert size. Estimates from ligation of only top largest cDNA fraction revealed the potential of making up 2.4 million independent clones with an average insert size of 1.9 kb and a median size of 2 kb.

[0118] The actual library screened consisted of 150,000 independent clones which were plated at a density of 1,500 clones/plate on 100 LB agar plates (to generate 100 pools of 1,500 clones/pool) with ampicillin at 50 µg/ml and grown overnight at 37°C. For purification of individual pools, each plate was overlaid with approximately 2 ml of Luria broth (LB), and the colonies were scraped off of each plate with a standard tissue culture cell scraper, and bacterial suspensions were transferred to microfuge tubes. Prior to purification, a glycerol stock was generated from each pool. Plasmid DNAs were purified using QIAprep spin columns (QIAGEN) according to manufacturer's instructions.

Transfections

[0119] CHO/P cells (Heffernan, M. and J.D. Dennis, *Nucleic Acids Res.*, 19:85-92 (1991)) were seeded into 24 well plates approximately 24 hours prior to transfection at a density of 40,000 cells/well. DNAs were transiently transfected using the LipofectAMINE™ reagent (GIBCO; Cat. #18324-012), essentially following the recommended protocol with further optimization for 24-well plates as follows: 200 ng of DNA (representing either a plasmid pool or purified control DNAs) was diluted to 20 µl with Opti-MEM 1 reduced serum media (GIBCO) and diluted into 20 µl of a mixture that consists of 18 µl Opti-MEM 1 and 2 µl of LipofectAMINE™ reagent. This liposome mixture was then incubated for approximately 30 minutes at ambient temperature after which, 200 µl of Opti-MEM 1 was added, and the entire mixture was then overlaid onto a well of CHO/P cells and returned to the incubator. After a 2.5 hour incubation at 37°C, 240 µl of MEM-α (Gibco) media with 20% fetal calf serum (FCS) was added to each well, and the cells were incubated for an additional 18-24 hours at 37°C. The media was then changed to standard MEM-α with 10% FCS, and the adhesion assay was performed approximately 20-24 hours later.

Adhesion assays for expression cloning

[0120] For the adhesion assays in the expression cloning screen, the murine T cell lymphoma TK1 which expresses high levels of α4β7 (Butcher, E.C., et al., *Eur. J. Immunol.*, 10: 556-561 (1980)) was used to detect CHO/P cells transfected with cDNAs capable of conferring an adhesive phenotype. TK1 cells were resuspended at a density of 2×10^6 /ml in an assay buffer which consisted of HBSS (Hanks Balanced Salt Solution, without Ca^{2+} or Mg^{2+}), supplemented with 2% bovine calf serum, 20 mM HEPES, pH 7.3, 2 mM Mg^{2+} , and 2 mM Ca^{2+} . Each well transfected with a DNA pool was preincubated with 0.25 ml of a combined supernatant containing monoclonal antibodies to both human VCAM-1 (MAb 2G7; Graber, N.T., et al., *J. Immunol.*, 145:819-830 (1990)) and murine MAdCAM-1 (MAb MECA-367; American Type Culture Collection (Rockville, MD), Accession No. HB9478; Streeter, P.R., et al., *Nature*, 331:41 (1988)); see also, U.S. Patent No. 5,403,919 to Butcher) in order to eliminate adhesion mediated by VCAM-1 (which is expressed at high levels in primate lymph nodes) or any potential contaminating murine MAdCAM-1 expression plasmids. After incubation at 4°C for 15 minutes, 0.25 ml of the TK1 cell suspension (5×10^5 TK1 cells) was added to each well, and incubation on a rocking platform was continued for an additional 30 minutes at 4°C. Plates were washed by gently inverting in a large beaker of phosphate buffered saline (PBS) followed by inversion in a beaker of PBS with 1.5% glutaraldehyde for fixation for a minimum of 1 hour. Wells were then examined microscopically (10X objective) for rosetting of TK1 cells.

Purification of macaque clones

[0121] Pools yielding one or more TK1 rosettes were further subfractionated by the following protocol: DNA representative of a positive pool was retransformed into DH10B and plated on ninety-six 100 mm petri dishes at a density of approximately 200 colonies/plate. Nitrocellulose filters were used to generate replica plates, and one set of each plate was then subjected to DNA purification and subsequent adhesion assays as described above. A replica plate representative of a positive pool was then further subfractionated into pools of 5 colonies, which were replica plated and grown overnight in LB media containing ampicillin. After one more round of DNA purification and adhesion assays, individual clones could then be grown up and the clones conferring adhesion of the TK1 cells were identified.

[0122] A full-length clone which was shown to encode MAdCAM-1 was obtained and designated clone 31D. Clone 31D, constructed in pSV-SPORT-1 (P25), contains a 5'-Sall to NotI-3' cDNA insert. Transformants of *E. coli* strain DH10B containing clone 31D were obtained. For expression in stable cell lines, this cDNA was subcloned into expression vector pcDNA-3 (Invitrogen), which carries a neo resistance gene suitable for G418 selection. In particular, insert of clone 31D was released by digestion with EcoRI (5') and NotI, and inserted into pcDNA-3 which had been cleaved with EcoRI and NotI to obtain pcD3pMAd.

Results

[0123] A cDNA expression library, divided into pools of 1,500 independent clones, was constructed from mRNA purified from macaque mesenteric lymph nodes (MLNs). Each pool was transiently transfected into the CHO/P cell line, and 48

hours after transfection, a cell adhesion assay was performed using the murine T cell lymphoma TK1. As VCAM-1 is expressed in MLNs, assays were done in the presence of anti-VCAM-1 MAb 2G7 (Grabner, N.T., et al., J. Immunol., 145: 819-830 (1990)). Additionally, assays were performed at 4°C in order to eliminate adhesion mediated by ICAM cDNAs (TK1 cells express high levels of LFA-1 and LFA-1 is not functional at 4°C). Microscopic examination of the assays revealed several wells with noticeable rosetting of TK1 cells. Two wells were chosen for further analysis by repeating the transfection and determining whether the binding mediated by the pools could be blocked by anti-β7 or anti-α4 MAbs. TK1 binding to one of the pools was completely inhibited by pre-incubation of TK1 cells with either anti-α4 MAb PS/2 or anti-β7 MAb FIB 504. This pool was subjected to three rounds of subfractionation until a single clone, called 31D, was isolated. Purified clone 31D mediated TK1 cell binding which could be inhibited by anti-α4 or anti-β7 antibodies.

[0124] The insert size of clone 31D was approximately 1.8 kb. Sequencing of the amino-terminus revealed several features consistent with a primate homolog of murine MAdCAM-1. The signal peptides were both 21 amino acids in length. Although the amino acid similarity was found to be only 48%, identity was 71% if non-conservative substitutions were considered. In addition, the protein encoded by clone 31D had a characteristic unique to Ig-family adhesion receptors: two pairs of cysteines separated by 3-4 (3 in this case) amino acids in the first immunoglobulin domain. Finally, 8 amino acids C-terminal to the first double cysteines is a stretch of 9 amino acids that is identical to a sequence in murine MAdCAM-1. Within this region was the sequence LDTSL, which aligns with a consensus motif for integrin/Ig family member interactions. Although this motif has general conservation with respect to other Ig adhesion receptors such as ICAM-1, ICAM-2, ICAM-3 and VCAM-1 (Osborn, L., et al., J. Cell. Biol., 124:601-608 (1994); Renz, M.E., et al., J. Cell. Biol., 125:1395-1406 (1994)), this exact sequence was previously found only in murine MAdCAM-1. The functional significance of this motif is suggested by the fact that a point mutation which changes the first L (leucine) of the motif at amino acid 61 to an R (arginine) in murine MAdCAM-1 had a dramatic effect on MAdCAM-1: α4β7 binding (not shown). The results of the functional studies together with these sequence characteristics indicate that clone 31D encodes a primate homolog to murine MAdCAM-1.

Screening of a human Phage library and purification of human clones

[0125] Human phage cDNA libraries were constructed in the λZiplox™ vector (Gibco/BRL). Human cDNA was made from RNA isolated from either normal or inflamed mesenteric lymph nodes (MLN) as described above. cDNA was synthesized as described above, ligated into the phage vector, and titered on bacterial strain Y1090 (ZL) ("ZL" = Ziplox). Duplicate filters from approximately 500,000 independent clones (50,000 clones/filter) from both the normal and the Crohn's MLN phage libraries were screened with ³²p-labeled full-length macaque MAdCAM-1 cDNA.

[0126] To prepare the probe, a -1.7 kb EcoRI-NotI fragment was excised from clone 31D, and isolated using GeneClean (BIO 101). The fragment was labeled with α³²P-dCTP by priming with random hexamers (Maniatis et al., In: Molecular Cloning (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1990)).

[0127] Screening conditions were as follows: 50,000 phage clones were plated on 150 mm petri dishes containing NZYCM agar (Gibco/BRL). After incubation ranging from 7-16 hours, the plates were overlaid with 132 mm nitrocellulose filters (Schleicher and Schuell, Keene, NH) for 2 minutes and then five minutes to transfer first and second (duplicate) lifts of phage clones, respectively. Filters were then soaked for 5 minutes in denaturing solution (1.5 M sodium chloride, 0.5 N sodium hydroxide) followed by neutralization in 1.5 M sodium chloride, 0.5 M Tris-HCl, pH 7.5. Filters were air dried for 15 minutes and then baked under vacuum for 2 hours at 80°C.

[0128] Filters were pre-hybridized for 2 hours at 55°C in 2M Na₂HPO₄, 0.5% SDS, 5X Denhardt's (1X Denhardt's solution is 0.02% bovine serum albumin, 0.02% ficoll, and 0.02% polyvinyl-pyrrolidone), 1 mM EDTA, and 50 μg/ml denatured salmon sperm DNA, and subsequently hybridized overnight at 55°C in the same buffer. Filters were washed once at room temperature in 2X SSC, 0.1% SDS (1X SSC is 0.15 M sodium chloride, 0.015 M sodium citrate), followed by three to four washes at 65°C in 0.1X SSC and 0.1% SDS. Filters were monitored with a Geiger counter to see that the background was reduced.

[0129] Positive clones were plaque purified, and the plasmid pZL1 containing the cDNA inserts was rescued using the CRE LOX recombination system (GIBCO) (plasmid pZL1 is contained within the body of the lambda Ziplox vector). In particular, a purified phage plaque was suspended in 200 μl of phage buffer (20 mM Tris HCl, pH 7.5, 145 mM NaCl, 8 mM MgSO₄ · 7H₂O, 0.01% gelatin) for 5 minutes at room temperature. 20 μl of the phage suspension was then added to 100 μl of an overnight culture of DH10B (ZL) and incubated for an additional 5 minutes. Dilutions of the mixture were then plated on LB plates supplemented with ampicillin at 50 μg/ml and 10 mM MgCl₂, and incubated overnight at 30°C. Single colonies, now containing the cDNA inserted into the pZL1 vector were grown as standard overnight cultures and plasmids were then purified using Qiagen plasmid purification reagents.

Identification of distinct functional human MAdAm-1 cDNA clones

[0130] Two human cDNA libraries from histologically normal human mesenteric lymph nodes, and inflamed mesenteric

lymph nodes from a patient with Crohn's disease were screened using the entire macaque MAdCAM-1 cDNA as a probe. One cross-hybridizing clone was isolated from the normal library, and two cross-hybridizing clones were isolated from the Crohn's library. One of the two clones isolated from the Crohn's library was about 1.3 kb, appeared to be incomplete at the 5'-end; and was not sequenced. The clone from the normal library (clone 4) was slightly larger (1624 bp) than the longer clone (1558 bp) isolated from the Crohn's library (clone 20). Although these two cDNAs differ in size by approximately 100 bp, their 5' and 3' untranslated sequences were almost identical, in length. Each clone appeared full-length, as they both contained an amino-terminal signal sequence that was almost identical to the macaque sequence.

[0131] Additionally, preliminary sequencing demonstrated the same distinguishing characteristics of the amino-terminal Ig-like domain as the primate cDNA. Since the differences in the size of these clones could not be attributed to the length of the untranslated sequences, it seemed likely that the variation resided in the coding region.

[0132] In order to determine whether each clone encoded functional human MAdCAM-1, the inserts of each clone were subcloned into the pCDNA-3 expression vector (Invitrogen, San Diego, CA), which carries a neo resistance gene suitable for G418 selection. The human cDNAs (which were made using NotI oligo-dT primers at the 3'-end, and Sall adapters at the 5'-end) were ligated into the λ ZipLox vector, which contains plasmid pZL1. pZL1 vectors with cDNA inserts were rescued as described above. For subcloning, the inserts of clones 4 and 20 were each released by digestion from the pZL1 backbone with EcoRI and NotI. The EcoRI-NotI (5'→3') fragments were isolated by GeneClean (Bio 101) following electrophoresis on a 1% agarose gel, and the fragments were ligated into pcDNA-3 which had been cleaved with EcoRI and NotI. The ligation mixture was used to transform a DH10B *E. coli* Max efficiency strain (GIBCO), and transformants were obtained following selection on LB agar supplemented with 50 μ g/ml ampicillin (Amp). Plasmids designated pcD3huMAd4 (insert from clone 4) and pcD3huMAd20 (insert from clone 20) were obtained and analyzed by restriction digestion.

[0133] Clone pcD3huMAd4 (insert from clone 4) or pcD3huMAd20 (insert from clone 20) was transiently transfected into CHO/P cells. Each clone directed the expression of a functional protein which could mediate binding and adhesion, as assessed by adhesion of CHO/P transfectants to the human B cell lymphoma RPMI 8866 (Figure 5) or to TK1 cells (not shown).

[0134] Adhesion of the CHO/P transfectants to RPMI 8866 cells was blocked by preincubation with anti- α 4 β 7 MAb ACT-1, but not by control IgG. Adhesion of transfectants to TK1 cells was blocked by anti- β 7 MAb FIB 504. These results indicate that clone 4 (from a normal mesenteric node library) and clone 20 (from a Crohn's library) each encode functional MAdCAM-1 proteins. To further characterize these distinct cDNAs, both clones were completely sequenced.

Results

[0135] The cDNAs from Clones 4 and 20, encoding human MAdCAM-1, are 1628 bp and 1543 bp, respectively, in length. cDNA from Clone 4 (Figure 1; SEQ ID NO:1) contains an open reading frame of 1218 bp encoding a predicted protein of 406 amino acids (SEQ ID NO:2), and a 3' untranslated region of 410 bp, but contains no 5' untranslated region. cDNA from Clone 20 (Figure 2; SEQ ID NO:3) contains 4 bp of 5' untranslated sequence, an open reading frame of 1146 bp encoding a predicted protein of 382 amino acids (SEQ ID NO:4), and a 3' untranslated region of 393 bp. The predicted molecular masses of the encoded proteins, after cleavage of a predicted signal sequence of 18 amino acids are 40,910 (clone 4) and 38,375 (clone 20) daltons.

[0136] Multiple alignments were performed to analyze the degree of similarity between the different cloned species of MAdCAM-1. Nucleotide alignments revealed 81.9% sequence similarity between mouse and rat MAdCAM-1 cDNAs, 41.8% similarity between mouse and macaque cDNAs, 42.1% similarity between murine and human (Clone 4) MAdCAM-1 cDNAs, and 41.8% similarity between murine and human (Clone 20) MAdCAM-1 cDNAs. Alignment of the nucleotide sequences of macaque MAdCAM-1 with human Clone 4 and Clone 20 cDNAs revealed sequence similarities of 70.7% and 75.0%, respectively.

[0137] The amino acid sequence similarities were determined to be 78.5% between mouse and rat MAdCAM-1, 44.3% between mouse and macaque, and 39% between murine and MAdCAM-1 encoded by human Clone 4.

[0138] Comparisons of cDNA clones 4 and 20 revealed a region which is homologous to the mucin domain of murine MAdCAM-1, due to a prevalence of serine, threonine and proline (69% for clone 4 and 76% for clone 20) residues (boxed in Figure 1 and Figure 2). This region, although similar in amino acid composition to murine MAdCAM-1, is highly divergent from murine MAdCAM-1. The domain is 71 amino acids long in clone 4, and 47 amino acids long in clone 20. This region also contains two polymorphisms: (1) a polymorphism at amino acid 240, which is proline (P) in clone 4 and serine (S) in clone 20; and (2) a polymorphism at amino acid 242, which is asparagine (N) in clone 4 and aspartate (D) in clone 20. In addition, the human mucin domains contain a repeat of 8 amino acids consisting of the sequence PPDTTS (Q/P)E, which appears eight times in clone 4 and five times in clone 20.

[0139] To assess the origin of clones 4 and 20, PCR primers flanking the repeat were used to amplify human genomic DNA. The following primers were used:

5'-CTC TAC TGC CAG GCC ACG-3' (Primer #1, SEQ ID NO:7)

5'-AGC CTG GGA GAT CTC AGG G-3' (Primer #2, SEQ ID NO:8)

5'-GCC ACG ATG AGG CTG CCT GG-3' (Primer #3, SEQ ID NO:9)

5'-GTG GAG CCT GGG CTC CTG GG-3' (Primer #4, SEQ ID NO:10)

The primers were nested primers. In the first reaction, primers 1 and 2 were used. For the second amplification reaction, a 1:1000 dilution of the first reaction was prepared, and 1 μ l was used with primers 3 and 4. Amplification reactions contained either 0.5 μ g of genomic DNA, 10 picograms of control plasmids (pcD3HuMAd4 or pcD3HuMAd20), or approximately 1 ng of double-stranded cDNA that was prepared previously for the ZipLox libraries. Genomic DNA was obtained from three sources (Promega; ClonTech, and by purification from Jurkat cells). The conditions of amplification were: one cycle for 5 minutes at 94°C; 25 cycles at 94°C for 45 seconds; 60°C for 45 seconds and 72°C for one minute followed by one cycle for 5 minutes at 72°C.

[0140] The amplification reactions from genomic DNA yielded two bands which comigrated with the individual products of PCR reactions using either clone 4 or clone 20 cDNA as template. This data suggests that the two cDNA clones are isoforms encoded by genomic DNA, and are probably generated by alternative splicing or by transcription of two different alleles. Extensive polymorphism and sequence divergence has been documented in other mucin sequences (e.g., Hilkens, J. et al., Trends, Biochem. Sci, 17: 359-363 (1992)). For example, repetitive portions of intestinal mucins are not well-conserved between rodents and humans (Gum, J.G. et al., J. Biol. Chem., 266: 22733-22738 (1991)). One caveat is that, based on an analysis of murine genomic structure, the human genomic DNA could contain an intron in this region. If so, the PCR primers used in this experiment would span the intron, and amplification of human genomic DNA would not be expected to produce bands of the same size as those produced by amplification of the cDNA controls. Isolation and analysis of human MAdCAM-1 genomic clones can conclusively exclude the possibility of a cloning artifact. Further assessment of normal and/or inflamed tissue from normal individuals and from patients with IBD, Crohn's disease or other inflammatory conditions can be performed to determine if there is a correlation between the clone 20 isoform and an inflammatory disease or activity.

[0141] The comparison of murine, macaque, and two isoforms of human MAdCAM-1 indicates that the amino-terminal portions of these receptors exhibit domain structures likely to be involved in recognition of α 4 β 7. In contrast, the regions of these receptors in a location corresponding to the location of the mucin/IgA domain of murine MAdCAM-1 display similar amino acid compositions (serine, threonine, proline-rich mucin regions), but are more divergent from one another.

Expression of human MAdCAM-1 RNA

[0142] Northern analysis was carried out using human multiple tissue Northern I and II (commercially prepared by Clontech, Palo Alto, CA), or 2 μ g of poly A+ RNA from cell lines and tissues that were prepared as described above. RNA was denatured and electrophoresed through a 1% agarose formaldehyde gel and transferred to a PVDF (Immobilon, Millipore) membrane by standard capillary blot procedures. RNA samples were stained with ethidium bromide to initially ensure that the quality and quantity of each cell or tissue RNA was equivalent. After transfer, RNA was fixed to membranes by UV crosslinking (Stratalinker, Stratagene) and this blot and the commercially prepared blots were pre-hybridized at 68°C for 1 hour in ExpressHyb (Clontech). The cDNA insert from clone 4 was labeled with α ³²P-dCTP by priming with random hexamers (Maniatis et al., In Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., (199)). Hybridization was performed at 68°C for 1 hour in ExpressHyb with denatured probe at a concentration of 2 X 10⁶ cpm/ml.

[0143] Blots were then washed in 0.1X SSC, 0.1% SDS for 60 minutes at 65°C with one change of wash at 30 minutes. The exposure time was 48 hours with an intensifying screen. After this exposure, the blot was stripped by washing for 10 minutes in 0.5% SDS and rehybridized under the same conditions with a β -actin cDNA. The exposure time was 2 hours.

Results

[0144] Northern blots were probed for MAdCAM-1 expression using the entire cDNA insert from clone 4 as a probe. A single RNA species of approximately 1.6 kb was highly expressed in the small intestine and was expressed to a lesser extent in the colon and spleen. No significant expression was observed in other tissues examined under these conditions, including thymus, prostate, ovaries, testes and peripheral blood leukocytes (PBL). This tissue-specific pattern of expression is consistent with studies in the mouse showing restricted expression of MAdCAM-1 in Peyer's Patches, MLN (mesenteric lymph node), intestinal lamina propria and some expression in the marginal sinus around splenic white pulp nodules in the spleen (Hemler, M.E., Annu. Rev. Immunol., 8:365 (1990); Berg, E.L., et al., Cellular and molecular mechanisms of inflammation, 2:111 (1991); Briskin, M.J., et al., Nature, 363:461 (1993)). No significant expression was observed in other tissues examined, including heart, brain, placenta, lung, liver, skeletal muscle, or kidney; however, low levels of expression were detected in pancreas. These data indicate that human MAdCAM-1 expression is tissue-specific with expression in mucosal tissues and spleen; a thorough immunohistochemical analysis of tissue distribution can be performed using monoclonal antibodies against human MAdCAM-1 (see below).

Example 2. Characterization of MAdCAM-1 ClonesFunctional adhesion assays5 *Plasmids:*

[0145] The following plasmids were used in the functional adhesion assays: (1) pSV-SPORT-1 (Gibco/BRL) or pcDNA-3 (Invitrogen) were used as controls; (2) murine MAdCAM-1 in pCDM8 (pCDMAD-7; Briskin, M.J., et al., Nature, 363: 461 (1993)); (3) seven domain human VCAM-1 (Polte, T., et al., Nucleic Acids Res., 18:5901 (1990)) in pcDNA3 (pCD3VCAM); and (4) human MAdCAM-1 in pcDNA-3 (pCDhuMAd4) (see above).

Monoclonal antibodies:

[0146] The following monoclonal antibodies (MAb) were used in the functional adhesion assays: (1) anti-murine MAdCAM-1 MAb MECA-367 (American Type Culture Collection (Rockville, MD), Accession No. HB9478; Streeter, P.R., et al., Nature, 331:41 (1988); and U.S. Patent No. 5,403,919 to Butcher); (2) anti-human VCAM-1 MAb 2G7 (American Type Culture Collection (Rockville, MD); Graber, N.T., et al., J. Immunol., 145:819-830 (1990)); (3) anti-murine $\alpha 4\beta 7$ MAb DATK 32 (Andrew, D.P., et al., J. Immunol., 153:3847-3861 (1994)); (4) anti-murine $\beta 7$ MAb FIB 504 (Andrew, D.P., et al., J. Immunol., 153: 3847 (1994)); (5) anti-human $\alpha 4\beta 7$ MAb ACT-1 (Lazarovits, A.I., et al., J. Immunol., 133: 1857 (1984)); (6) anti-human integrin $\beta 1$ (CD29) (Becton Dickinson; San Jose, CA, Cat. #550034); and (7) murine IgG1 and rat IgG2A as irrelevant controls.

Cell Lines:

[0147] The following cell lines were used in functional adhesion assays:

[0148] (1) Murine T cell lymphoma TK1 (Butcher, E.C., et al., Eur. J. Immunol., 10:556-561 (1980); E. Butcher (Stanford, CA); (2) RPMI 8866, a human B cell lymphoma line which expresses $\alpha 4\beta 7$ (and not $\alpha 4\beta 1$) (American Type Culture Collection (Rockville, MD); Erle, D.J., et al., J. Immunol., 153:517 (1994); a gift from D. Erle); (3) JURKAT, a human T cell line which expresses $\alpha 4\beta 1$ (and not $\alpha 4\beta 7$) (American Type Culture Collection (Rockville, MD)); and (4) Ramos, a human (B lymphocytic) Burkitt lymphoma cell line that expresses $\alpha 4\beta 1$ (and not $\alpha 4\beta 7$) (American Type Culture Collection (Rockville, MA), Accession No. ATCC CRL 1596).

Functional adhesion assays:

[0149] For functional adhesion assays, plasmids encoding various species of MAdCAM-1, human VCAM-1, and control plasmids were introduced by transient transfection into CHO/P cells as described above (Example 1) with the following modifications. As several wells were to be transfected for antibody inhibition studies, a master liposome mix with multiples of the wells to be transfected was first made for each plasmid. This ensured that the same liposome mixture was transfected into each well.

[0150] 48 hours after transfection, the medium was removed. An antibody supernatant (0.25 mls) (containing either anti-human VCAM-1 MAb 2G7 or anti-murine MAdCAM-1 MAb MECA-367), or 0.25 mls of adhesion assay buffer as a control were added, and the mixture was preincubated at 4°C for 15 minutes.

[0151] In parallel, lymphocyte cell lines (RPMI 8866 or Jurkat) were spun down and resuspended at a density of 2×10^6 /ml in assay buffer consisting of HBSS (without Ca^{++} or Mg^{++}) supplemented with 2% bovine calf serum, 20 mM HEPES pH 7.3, 2 mM Mg^{++} and 2 mM Ca^{++} . 0.25 ml aliquots (5×10^5 cells) of these RPMI 8866 or JURKAT cell suspensions were preincubated with a small volume of various purified antibodies or with an equal volume of DATK 32 supernatant at 4°C for 15 minutes. Where DATK 32 was used in a preincubation with a cell line, prior to the start of the assay, the supernatant or buffer present in the wells (containing the transfectants) was aspirated in order to obtain volume of 0.5 ml total for the adhesion assay.

[0152] For preincubations, purified antibodies (ACT 1, FIB 504 anti- $\beta 1$) and control IgG antibodies were used at concentrations of 20 $\mu\text{g}/\text{ml}$. 0.25 mls of antibody supernatant (used neat) containing anti-human VCAM-1 (MAb 2G7) or anti-murine MAdCAM-1 (MAb MECA-367) were used in preincubations. 0.25 mls of antibody supernatant of DATK 32 were used in the preincubation.

[0153] After the preincubations, cell lines (Jurkat or RPMI 8866) were combined with the transfectants in the wells, and incubation on a rocking platform was continued for an additional 30 minutes at 4°C.

[0154] Assays were fixed as described above. Plates were washed by gentle inversion in a large beaker of phosphate buffered saline (PBS), followed by inversion in a beaker of PBS with 1.5% glutaraldehyde for fixation for a minimum of 1 hour. Adhesion was assessed by counting both lymphocytes and CHO cells in a field at 20X magnification. For each

assay, the number of lymphocytes bound per CHO/P cell was averaged as a minimum of four fields with standard error. Results in each case are from one of three experiments performed with similar results.

Results

[0155] Murine MAdCAM-1 specifically binds lymphocytes expressing $\alpha 4\beta 7$ (and not $\alpha 4\beta 1$). In order to determine the specificity of human MAdCAM-1 lymphocyte interactions, adhesion assays were performed to assess the ability of transiently transfected CHO/P cells expressing human MAdCAM-1 to bind to the RPMI 8866 cell line which only expresses $\alpha 4\beta 7$ (Erle, D.J., et al., J. Immunol., 153:517 (1994)), or to the T cell line Jurkat, which exclusively express $\alpha 4\beta 1$. Binding of these cell lines was compared to that of transiently transfected CHO/P cells expressing murine MAdCAM-1 and human VCAM-1. The results are presented in Figures 4A-4B.

[0156] RPMI 8866 cells did not bind to control transfectants, but avidly bound to transfectants expressing human or murine MAdCAM-1. This binding was completely inhibited by preincubation with anti- $\alpha 4\beta 7$ MAb ACT-1 (Figure 4A). VCAM-1 transfectants failed to bind RPMI 8866, which is consistent with the previous demonstration that $\alpha 4\beta 7$ /VCAM-1 interactions are activation-dependent (Postigo, A.A., et al., J. Immunol., 151:2471-2483 (1993); Ruegg, C., et al., J. Cell. Biol., 117:179-189 (1992)). The failure of RPMI 8866 cells to bind VCAM-1 transfectants was not due to lack of expression, as FACS analysis using anti-VCAM-1 MAb 2G7 indicated a transfection efficiency of ~ 60%. Moreover, the same VCAM-1 transfectants were able to bind Jurkat cells, and binding was completely inhibited by preincubation with either anti-VCAM-1 or anti- $\beta 1$ MAbs (Figure 4B). Murine and human MAdCAM-1 transfectants did not bind Jurkat cells (an $\alpha 4\beta 1$ positive line). These data demonstrate that human MAdCAM-1 can selectively bind to human leukocytes lymphocytes expressing $\alpha 4\beta 7$ integrins.

L1-2 and CHO cell transfectants

[0157] The mouse L1-2 cell line is derived from a pre-B lymphoma, and was obtained from Dr. Eugene Butcher (Stanford University, Stanford, CA). The genes encoding either the macaque or human cDNAs for MAdCAM-1 were subcloned into the pcDNA-3 vector (Invitrogen) as described above. The resulting plasmids (pcD3HuMAd4, pcD3HuMAd20, or pcD3PMad (macaque)) were introduced into L1-2 cells by transfection as follows: L1-2 cells were grown to a density of approximately 10^6 /ml. Either 50, 25 or 12.5 million cells were washed in HBSS and then resuspended in a 0.8 ml of a buffer consisting of Hanks balanced salt solution supplemented with 20 mM HEPES, pH 7.05. A solution consisting of 20 μ g of linearized plasmid, 500 μ g of tRNA and HBSS to bring the final volume to 200 μ l was added to the cell suspension to bring the total volume to 1ml. After a 10 minute incubation at room temperature the cell/DNA mixture was transferred to an electroporation cuvette (BioRad, Richmond, CA) and electroporated at 250 volts, 960 mF in a BioRad gene pulser. Following another 10 minute incubation at room temperature, the cells were diluted to 25 ml in standard L1-2 growth media (RMPI 1640, 10% Hyclone fetal bovine serum, 50 U/ml Penicillin/Streptomycin (Gibco) and 0.29 mg/ml L Glutamine (Gibco) and returned to the incubator at 37°C. 48 hours later, the cells were pelleted by centrifugation and resuspended in 50 ml of L1-2 media supplemented with G418 (Geneticin; Gibco) at 0.8 mg/ml. Dilutions of the cell suspension were plated in 96-well microtiter plates and single colonies were grown up analyzed for expression of MAdCAM-1.

[0158] L1-2 cell clones expressing MAdCAM-1 could be detected by adherence to TK1 cells. L1-2 (non-transfected cells) and TK1 cells both grow as single cell suspensions. Surface expression of MAdCAM-1 can be detected by its ability to mediate adhesion by virtue of its interaction with $\alpha 4\beta 7$ expressed on TK1 cells. Specificity of this interaction was further demonstrated by inhibition by pretreatment of TK1 cells with anti- $\beta 7$ MAb FIB 504.

[0159] CHO cells (Chinese Hamster Ovary Cells; American Type Culture Collection (Rockville, MD)) stably transfected with either the macaque or human MAdCAM-1 clones were prepared by electroporation as described above for the L1-2 cells with the following exceptions. Media for CHO cell growth was α -MEM with deoxyribonucleosides (Gibco) and 10% fetal calf serum (Gibco) and 50 U/ml Penicillin/Streptomycin (Gibco) and 0.29 mg/ml L Glutamine (Gibco). Selection media consisted of the same media with 0.55 mg/ml G418 (Gibco). Single clones were grown up and analyzed for their ability to exhibit $\alpha 4\beta 7$ -dependent binding of RPMI 8866 cells using the functional adhesion assay described above (for transients), except that cells were plated at 50,000 cells per well in a 24-well plate the day before the assay. Using this criteria, a line called CHO HuMAd 4 was established.

Monoclonal antibodies capable of inhibiting adhesion

[0160] Monoclonal antibodies against human MAdCAM-1 were generated by immunizing C57BL/6 mice with L1-2 MAdCAM-1 transfectants. Mice were immunized intraperitoneally with 10 million cells resuspended in HBSS three times at two week intervals, and a final fourth immunization (of 10 million cells resuspended in HBSS) was injected intravenously. The first immunization was performed with a mixture of two clones (L1-2 cell clone 23 and clone 19) expressing macaque

MAdCAM-1. The remaining boosts were done with a single L1-2 clone (L1-2 clone HuMAD4/17) expressing human MAdCAM-1.

[0161] A successful fusion was performed which generated approximately 5,000 hybridomas. Four days after the final intravenous injection, the spleen was removed and a single cell suspension was prepared in serum free DMEM media. These cells were fused with the fusion partner SP2/0, according to the method of Galfre *et al.* (Galfre, G., et al., Nature, 299:550-552 (1977)). 20 ml of spleen cells and 20 ml of SP2/0 cells were combined, spun at 800 g for 5 minutes and the media was removed by aspiration. A solution of 50% polyethylene glycol 1500 (PEG 1500) (Boehringer Mannheim, Indianapolis, IN) prewarmed to 37°C was added to the cell pellet over 2 minutes, followed by 10 ml of DMEM media over 3 minutes. The cell suspension was spun at 600 g for 3 minutes and the supernatant was removed. The pellet was resuspended gently in DMEM media containing 20% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin sulfate, and HAT selection media (Sigma, St. Louis, MO). Cells were plated into ten 96-well flat bottom microtiter plates at 200 µl/well.

[0162] Ten days after the fusion, supernatants from the wells were screened for reactivity against CHO human MAdCAM-1 transfectants (CHO HuMAd 4 cells), by fluorescence staining. Staining of 500,000 cells per sample was performed essentially as described, using 50 µl of each supernatant and 50 µl cells (E. Harlow and D. Lane, 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). The secondary antibody was an FITC-labeled anti-murine IgG (H + L) (Jackson Labs) that was diluted 1:200. Strong reactivity was judged as a 2-3 log increase in fluorescence of as compared with untransfected CHO cells.

[0163] 48 antibody supernatants were selected for strong reactivity against CHO HuMAd 4 cells. These antibody supernatants were then screened for their ability to block the adhesion of CHO HuMAd 4 cells to RPMI 8866 cells. As a control, the ability of supernatants to inhibit Ramos cell binding to VCAM-1 transfectants was examined, as it should not be affected by a specific anti-human MAdCAM-1 MAb. To identify blocking anti-human MAdCAM-1 monoclonal antibodies, the following assay was performed. To provide control transfectants, CHO/P cells were transfected with pCD3VCAM as described above, and were assayed 48 hours after transfection. 48 hours before the adhesion inhibition assay, 40,000 cells per well of VCAM-1 transient transfectants were plated into 24 well plates. 24 hours before assay, 50,000 cells per well of CHO HuMAd 4 transfectants were plated in 24 well plates. On the day of the assay, each anti-human MAdCAM-1 supernatant (0.25 mls) was added to a well containing either CHO HuMAd 4 transfectants or VCAM-1 transfectants, and the mixture was preincubated at 4°C for 15 minutes. Adhesion assays were performed, using (1) RPMI 8866 cells with the MAdCAM-1 transfectants or (2) Ramos cells (a human B cell line that expresses α4β1) with the VCAM-1 transfectants.

[0164] In parallel, cells (RPMI 8866 or Ramos) were resuspended at a density of 2×10^6 /ml in an assay buffer consisting of HBSS (without Ca⁺⁺ or Mg⁺⁺) supplemented with 2% bovine calf serum, 20 mM HEPES pH 7.3, 2 mM Mg⁺⁺ and 2 mM Ca⁺⁺. After the preincubation of the transfectants with antibody, 0.25 mls of the RPMI 8866 or Ramos cell suspensions (5 x 10⁵ cells) were added to each well, and incubation on a rocking platform was continued for an additional 30 minutes at 4°C. The wells were washed, fixed and examined as described above to assess inhibition of binding.

[0165] Eleven out of 48 of the hybridoma supernatants examined displayed substantial blocking activity, inhibiting the adhesion of RPMI 8866 cells to transfectants expressing MAdCAM-1. Adhesion of Ramos cells to transfectants expressing VCAM-1 was unaffected, indicating selective inhibition of α4β7-mediated interactions. Selected blocking hybridomas were subcloned by limiting dilution.

Results

[0166] Stable cell lines expressing macaque or human MAdCAM-1 were made in the murine pre-B lymphoma L1-2. These cells were used to immunize C57BL/6 mice and prepare hybridomas. The resulting fusion was screened by immunofluorescence staining of CHO HuMAd 4 transfectants expressing human MAdCAM-1. Screening of approximately 1,000 wells produced 48 supernatants exhibiting strong reactivity against the CHO HuMAd 4 transfectants, while non-transfected CHO cells were negative. These supernatants were subsequently tested for their ability to specifically block adhesion of RPMI 8866 cells to human MAdCAM-1 transfectants.

[0167] 11 of the 48 hybridoma supernatants examined could specifically inhibit the adhesion of RPMI 8866 cells to MAdCAM-1, while adhesion of Ramos cells (which express α4β1) to VCAM-1 transfectants was unaffected by the same supernatants. These hybridomas were designated 10G4, 8C1, 10G3, 9G12, 9E4, 7H12, 10F2, 10A6, 1E5, 2F5, 7G11.

Example 3. Design and functional analysis of a human MAdCAM-1-IgG chimera

Construction of MAdCAM-IgG Chimera

[0168] Human MAdCAM-1 clone 4 cDNA in pCDNA3 (Invitrogen, San Diego, CA.), called pcD3huMAd4 (Example 1) was used as a template for PCR amplification of extracellular regions of human MAdCAM-1 to be fused with the constant

region of human IgG1. Primer HUMADIG4/2 (SEQ ID NO:11), which contains the 5' end of human MAdCAM-1 coding sequence (ATG codon, bold), was synthesized:

HindIII
5' - **GGAAGCTT**CCACCATGGATTTCTGGACTGGCCC - 3'

This 5' primer was used in conjunction with a 3' primer designated HUMADIG2 (SEQ ID NO:12) to amplify regions encoding the two amino-terminal immunoglobulin-like (Ig) domains of human MAdCAM-1. Primer HUMADIG2 (SEQ ID NO:12), which contains a portion complementary to coding strand nucleotides 667-683 of SEQ ID NO:1, has the following sequence:

SpeI
5' - CCGACTAGTGTCGGGCTGTGCAGGAC - 3'

Alternatively, the 5' primer was used in conjunction with 3' primer HUMADIG3 to amplify a region encoding the entire extracellular domain of human MAdCAM-1 (clone 4). The 3' primer HUMADIG2 (SEQ ID NO:12), which contains a portion complementary to coding strand nucleotides 992-1010 of SEQ ID NO:1, has the following sequence:

SpeI
5' - **GGACTAGT**GGTTTGGACGAGCCTGTTG - 3'

The primers were designed with a 5' HindIII site or 3' SpeI sites as indicated. These primers were used to PCR amplify two different MAdCAM fragments, using a PCR optimizer kit from Invitrogen (San Diego, CA). The PCR products were digested with the enzymes HindIII and SpeI to generate ends for cloning. The products were subsequently purified by gel electrophoresis using the Glassmax DNA isolation system (Gibco, Bethesda MD).

[0169] A ~1 kb fragment encompassing the CH1, H (hinge), CH2 and CH3 regions was excised by digestion with SpeI and EcoRI from a construct encoding a human immunoglobulin γ 1 heavy chain having an Fc-mutated human constant region. The antibody encoded by this construct was used as an isotype matched irrelevant control hereinbelow. The human constant region in this construct was originally obtained by PCR amplification of the CAMPATH-1H heavy chain (Reichmann, L. et al., Nature, 322: 323-327 (1988)) as described by Sims, M.J. et al. (J. Immunol., 151: 2296-2308 (1993)) and Waldmann et al. (WO 93/02191, February 4, 1993 (page 23)), the teachings of which are each incorporated herein by reference in their entirety. The mutations in the constant region of this construct (Leu²³⁴ → Ala²³⁴ and Gly²³⁷ → Ala²³⁷) were designed to reduce binding to human Fc γ receptors, and were produced by oligonucleotide-directed mutagenesis. Thus, the MAdCAM-Ig fusions produced contain the SpeI-EcoRI constant region fragment described by Sims et al. (J. Immunol., 151: 2296-2308 (1993)) and Waldmann et al. (WO 93/02191), except for the introduction of Leu²³⁴ → Ala²³⁴ and Gly²³⁷ → Ala²³⁷ mutations.

[0170] The 1 kb SpeI-EcoRI fragment encoding the Fc-mutated IgG1 constant region was isolated by gel electrophoresis using the Glassmax DNA isolation system (Gibco, Bethesda MD). This constant region fragment, the HindIII-SpeI fragments containing either (a) the two N-terminal Ig domains of MAdCAM-1 or (b) the entire extracellular domain, were ligated in a three-way ligation to vector pEE12 (Stephens, P.L. and M.L. Cockett, Nucl. Acids Res., 17: 7110 (1989)) and Bebbington, C.R. and C.C.G. Hentschel, 1987, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells, (Academic Press, N.Y.), which had been digested with HindIII and EcoRI. Transformants of the bacterial strain DH10B were obtained. Colonies were grown and mini plasmid preps were analyzed by restriction mapping. Three constructs which encode fusion proteins comprising either the entire extracellular domain of MAdCAM-1 (construct HuMAdIg21) or the two N-terminal Ig domains (construct HuMAdIg31 or HuMAdIg38) fused to the Fc-mutated IgG1 constant region, were sequenced across the entire MAdCAM-1 portions, confirming proper fusion of segments and the absence of PCR induced mutations. For initial testing, each construct was transiently transfected onto monolayers of 5 X 10⁷ COS cells in 1 ml of RPMI buffer (no serum) and 25 μ g of plasmid using electroporation with a Biorad Gene Pulser under standard conditions (960 μ F, 250 V). 72-96 hours after transfection, supernatants were harvested, passaged through 0.45 μ filters and stored at 4°C in the presence of 0.05% sodium azide. Production of chimera was confirmed by a sandwich ELISA, using an anti-human IgG1 antibody as capture antibody and the same antibody, which was conjugated to alkaline phosphatase as second antibody for detection. Irrelevant control antibody

(having an identical constant region) was used as a standard. The chimera was also analyzed by western blotting using an anti-human MAdCAM-1 monoclonal antibody, and was found to run at approximately 200 kd, consistent with the size of a homodimer.

Soluble human MAdCAM-Ig chimeras specifically bind $\alpha 4\beta 7$ positive cells

[0171] Supernatants from four different transfections were assayed for their ability to stain the T cell line HuT 78, which was previously shown to bind MAdCAM-1 only in the presence of Mn^{++} . Accordingly, each solution used in this assay contained 2 mM Mn^{++} . HuT 78 cells (a human T cell lymphoma line; American Type Culture Collection, Accession No. ATCC TIB 161) are $\alpha 4\beta 7$ -bearing cells. To test the binding specificity of the chimeras, Hut 78 cells were preincubated with either media alone (RPMI 1640 with 2% FCS) or media and 10 $\mu g/ml$ of the anti- $\beta 7$ antibody FIB 504. Approximately 100,000 cells were incubated on ice for 15 minutes and then washed with HBSS plus 2% FCS / 2 mM Ca^{++} / 2 mM Mn^{++} . Cells were then incubated for 20 minutes on ice with media once again or with supernatants from one of four independent transfections, two with a chimera containing the entire extracellular domain of MAdCAM-1 (clone 21) and two with a truncated form of MAdCAM containing the two N-terminal Ig domains (clone 38) for 20 minutes. After washing, cells were then incubated with an anti-human IgG antibody conjugated with phycoerythrin and staining above background was assessed by flow cytometry (FACScan). Only cells incubated with the chimera supernatants stained above background, while preincubation with the $\beta 7$ MAb reduced this staining to background levels, indicating a specific interaction of the chimera with the $\alpha 4\beta 7$ integrin (Figures 17A-17E).

[0172] Permanent NSO cell lines secreting human MAdCAM-Ig chimera were selected after transfection by electroporation, by growth in a glutamine free media as previously described (Cockett, M.L., et al., Bio/Technology, 8: 662-667 (1990)). Cloned lines were adapted to growth in spinner culture. Supernatants from three of these cloned lines (samples B-D), and a partially purified chimera (Clone 21, purified by binding to protein A, sample A) were tested for their ability to support adhesion of the B cell line RPMI 8866. Briefly, NEN maxisorb plates were incubated with 100 μl /well of Protein A at 20 $\mu g/ml$ in carbonate buffer, pH 9.5 overnight at 4°C. Plates were then washed 2X with RPMI 1640 media (no serum). 100 μl of chimera (or serial dilutions in RPMI) were bound to the wells at 37° for 2 hours and then washed once. Wells were then blocked with FCS for 1 hour at 37°C, washed once, and then preincubated with tissue culture supernatants containing either an anti-human VCAM-1 MAb (2G7) as a control or the anti-human MAdCAM-1 MAb 10G3 (Example 2). 2G7 and 10G3 MAbs were removed before addition of cells. RPMI 8866 cells were fluorescently labeled by preincubation with BCECF-AM stain (BCECF-AM; 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester; Molecular Probes), 100 μl of cells were added to each well (to a final concentration of 10^5 cell/well), and incubated on a rotary shaker for 30 minutes at room temperature. Binding of RPMI 8866 cells to immobilized chimeras was assessed by reading fluorescence values using a Fluorescence Concentration Analyzer (IDEXX). Specific binding was demonstrated as only the anti-human MAdCAM-1 MAb could block binding of cells to MAdCAM-Ig chimera (Table 1).

[0173] These and other such chimeric fusion proteins can be used for assessing the ability of an agent (e.g., small molecule) to block $\alpha 4\beta 7$ binding to chimera, to identify inhibitors of $\alpha 4\beta 7$ -MAdCAM interaction. Additionally, since chimeric fusion proteins can bind to $\alpha 4\beta 7$ positive lymphocytes in solution, they provide candidate inhibitors of *in vivo* lymphocyte recruitment to inflammatory sites.

TABLE 1

Sample	Anti HuMAd MAbs	Neat	1:2	1:4	1:8	1:16	1:32
<u>A</u>	-	195527	195527	195527	18560	4254	3558
	+	3860	3092	1746	2200	482	564
<u>B</u>	-	195527	195527	195527	195528	52056	2932
	+	6526	3626	3274	2978	1648	1518
<u>C</u>	-	195527	195527	35548	9570	21782	1926
	+	4566	4094	3922	3492	2436	2566
<u>D</u>	-	195527	30840	46852	16270	5474	2656

(continued)

Sample	Anti HuMAd MAbs	Neat	1:2	1:4	1:8	1:16	1:32
	+	7350	6794	6020	4510	6548	5122
5	The $\alpha 4\beta 7$ Positive B Cell Line RPMI 8866 Specifically Binds Soluble Human MAdCAM Ig Chimera. The Human MAdCAM-1 Ig Chimera Clone 21 that was partially purified over protein A (Sample A) or tissue culture supernatants from different NSO clones (Samples B-D) were immobilized on 96 well plates with protein A, and either preincubated with an anti-VCAM-1 MAB 2G7 (designated "-" under MAbs) as a negative control or with the anti-human MAdCAM MAB 10G3 ("+").						
10	Purified chimera or supernatants (used either undiluted ("neat") or at serial 1:2 dilutions) was bound to wells via Protein A, and incubated with fluorescently-labeled RPMI 8866 cells on a rotary shaker for 30 minutes at room temperature. After washing with an automated plate washer (EL 404 Microplate Autowasher, BIO-TEK Instruments), bound cells were counted with an automated plate reader (IDEXX). Raw numbers are thus a reflection of numbers of cells bound.						
15							

Example 4. Inhibition of Lymphocyte Recruitment to colonA. DSS-Induction of colitis in mice

[0174] BALB/c mice were given access to a 5% solution of dextran sodium sulfate (DSS) in their drinking water for a period of 10 days, as previously described (Lab. Invest. 69:238-249, 1993). During this time period, the mice developed clinical symptoms of colitis including softening of stools and bloody diarrhea. Multifocal epithelial injury and ulceration, similar to ulcerative colitis in humans, was evident on histologic examination of colonic mucosa from affected mice. Moreover, affected mice lost 20-30% of their initial body weight by day 10.

Antibody blockade of $\beta 7$ and MAdCAM interactions

[0175] To determine the efficacy of $\beta 7$ -specific antibodies in blocking the recruitment of lymphocytes to the colon, BALB/c mice were given daily intraperitoneal (i.p.) injections of 100 μ g of monoclonal antibodies against $\beta 7$, consisting of either FIB21 or FIB30 in saline, as previously characterized and described (Berlin, C., et al., Cell 74:185-195, 1993; Michie, S.A., et al., Am. J. Pathol. 143:1688-1698, 1993; Hamann, A., et al., J. Immunol. 152:3282-3293, 1994) or an isotype-matched control rat monoclonal antibody at the same dose (Andrew *et al.*, *supra*) over the 10 day course of DSS treatment.

Methods of evaluation

[0176] Two methods were used to evaluate efficacy of the antibody therapy to inhibit leukocyte infiltration and mucosal injury in the colitic mouse. In the first method, treatment was judged histologically by two blinded observers using a scoring system for the evaluation of epithelial injury and degree of leukocyte cellular infiltration (Table 2). For this assessment, colon tissue was first fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned, and the sections were stained with hematoxylin and eosin prior to examination.

TABLE 2: PATHOLOGY EVALUATION

Grade	Definition
<i>INFLAMMATION</i>	
Normal (0)	Absence of clusters of polymorphonuclear leukocytes (PMNs) or mononuclear cells in the lamina propria; absence of intraepithelial PMNs
Mild (1)	Focal aggregates of PMNs and/or mononuclear cells in the lamina propria (equivocal or slight) or presence of isolated intraepithelial PMNs in 3 or fewer crypts per cross-section
Moderate (2)	Focal aggregates of PMNs and/or mononuclear cells in the lamina propria (multi-focal or diffuse 2-5X) or intraepithelial PMNs in more than 3 crypts per cross-section

(continued)

Grade		Definition
<i>INFLAMMATION</i>		
5	Severe (3)	Diffuse infiltration of PMNs or mononuclear cells in the lamina propria (diffuse >5X) or crypt abscesses
<i>STRUCTURAL OR EPITHELIAL ALTERATIONS</i>		
	Normal (0)	Tight crypts, no erosion, columnar epithelial cells
10	Mild (1)	Epithelial immaturity; equivocal irregularity of epithelial surface
	Moderate (2)	At least two foci of crypt branching or loss of crypts (<50%); loss of surface epithelium
15	Severe (3)	Diffuse or multifocal branching or loss of crypts (>50%); fibrosis; complete loss of epithelium (focal)

[0177] Additional histologic assessment was performed using immunohistochemistry for the detection and semiquantification of lymphocytes expressing $\beta 7$ integrins and mucosal venules expressing MAdCAM. As previously described (Ringler, D.J., et al., Am. J. Pathol., 134: 373-383 (1989)), colon tissue was first snap-frozen in OCT compound, sectioned while frozen, and the sections were subsequently fixed in acetone for 10 min at 4°C. After washing in phosphate buffered saline (PBS), nonspecific antibody binding sites were blocked with 10% normal rabbit serum diluted in PBS for 10 min, followed in sequence with washes by FIB21 antibody at 20 μ g/ml in PBS for 30 min at room temperature (RT), biotinylated rabbit anti-rat polyclonal antibody, avidin-peroxidase complexes, and finally the chromogen, diaminobenzidine and hydrogen peroxide diluted in Tris buffer.

[0178] In the second method, recruitment of lymphocytes to the colon was quantitatively assessed using radiolabeled mesenteric lymph node lymphocytes from syngeneic donor mice. The experimental design of the animal experiments was similar to that described above except that BALB/c mice were placed on 5% DSS for 9 days (instead of 10) and on day 8, mice were given i.p. injections of 100 μ g of FIB21 (anti- $\beta 7$), MECA-367 (anti-MAdCAM), a mixture of both, or an isotype-matched control monoclonal antibody in saline. On day 9, mesenteric lymph node cells were isolated from donor syngeneic BALB/c mice, labeled with ^{51}Cr , and 5.0×10^6 cells/mouse were incubated for 30 minutes at 37°C with 500 μ g control antibody, 250 μ g of MECA-367, 500 μ g FIB21, or both (total amount is 750 μ g) in saline. The labeled cells and antibody were then injected intravenously (i.v.) into the DSS-treated recipient mice. Full-length colons were harvested from all experimental animals 1 hour after injection, and γ -irradiation was measured using a γ -counter.

Data analysis

[0179] Differences between mean scores obtained for each group of animals were assessed for statistical significance using a paired Student's t-test. Differences between means were considered significant when $P < 0.05$.

Results

[0180] Histologically, inflammation and epithelial injury to the mucosa were most severe in the descending colon, rectum and cecum. Analysis of frozen tissue sections of colon by immunohistochemistry revealed that the most significant recruitment of $\beta 7^+$ lymphocytes was to the right colon. In addition, the level of expression of the mucosal vascular addressin, MAdCAM-1, was found to be expressed only at low levels in vessels in the intestinal mucosa early in DSS treatment (3 days), but increased dramatically after 9 days of DSS treatment, supporting the conclusion that $\beta 7$ and MAdCAM-1 interactions are relevant to the inflammatory process in the colonic mucosa during DSS-induced colitis.

[0181] Histologic evaluation of mice exposed to a 10-day course of DSS and daily therapy using $\beta 7$ -specific antibodies demonstrated that substantial reductions of leukocyte recruitment ($P < 0.01$ for FIB30 and $P < 0.001$ for FIB21) and epithelial injury ($P < 0.05$) occurred in right (ascending) colon compared to animals receiving a control antibody at the same dose (Figures 7A and 7B). Furthermore, analysis using immunohistochemistry of frozen sections from these animals suggested that the number of $\beta 7^+$ cells recruited to the right colon, but not other sections of colon, during DSS treatment was reduced.

[0182] Lymphocyte recruitment to inflamed colon was then quantitatively assessed using radiolabeled mesenteric lymphocytes taken from syngeneic donors. One hour after injection of these cells in DSS-treated recipients, there was a trend towards a reduction in the number of ^{51}Cr -labeled cells recruited to colon in mice that were treated with either $\beta 7$ -specific antibodies or the MAdCAM-specific antibodies, but not in mice treated with the isotope-matched control

antibodies (Figure 8).

B. Induction of colitis in *scid* mice and inhibition of recruitment of lymph node cells to colon

[0183] *Scid* mice reconstituted with CD45RB^{hi} CD4⁺ T cells develop colitis and a severe wasting syndrome. The colitis that develops in *scid* mice reconstituted with CD45RB^{hi} CD4⁺ T cells differs from most other murine models of IBD in that the induced colitis in the *scid* mouse clearly requires the presence of CD4⁺ T cells for the induction, if not the pathogenesis, of the disease (Powrie, Immunity, 3:171 (1995), the teachings of which are incorporated herein in by reference their entirety)).

[0184] A modification of the method of Morrissey *et al.* and Powrie *et al.* (Morrissey *et al.*, J. Exp. Med., 178:237 (1993); Powrie *et al.*, Int. Imm., 5:1461 (1993), the teachings of which are both incorporated herein by reference in their entirety) was used to enrich for CD4⁺ T cells, isolated from BALB/c spleen, by depletion of granulytic leukocytes, CD8⁺ T cells, B220⁺ cells, I-A⁺ cells and MAC-1⁺ macrophages. CD45RB^{hi} cells were selected by cell sorter, gating on the brightest 40-45% of CD4⁺ cells stained with anti-CD45RB. Recipient *scid* mice were reconstituted by intravenous (i.v.) injection of 1 X 10⁶ CD45RB^{hi} or CD45RB^{lo} T cells into the tail vein. Four mice were reconstituted with CD45RB^{hi} T cells and four mice with CD45RB^{lo} T cells.

[0185] Reconstituted mice were monitored weekly for changes in weight and the development of fecal occult blood. Typically, within 4-6 weeks post reconstitution, the difference in body weight of mice reconstituted with CD45RB^{hi} T cells relative to control *scid* mice reconstituted with an equal number of CD45RB^{lo} T cells, became statistically significant (Figure 18).

[0186] Colitis can be induced in this model with as few and 5 X 10⁴ cells. Generally, from 1 - 5 X 10⁵ is used. Although the kinetics of onset of disease is not uniform among mice in a given reconstitution, the colitis is of similar severity once the body weight has decreased to 75-85% of initial weight. Histological observations were consistent with the reports of others, and indicate that the colitis in this model is characterized by massive infiltration of CD4⁺ T cells in the mucosa and sub-mucosa, epithelial immaturity, ulceration, crypt hyperplasia, loss of goblet cells and crypt abscesses. Similar to Crohn's Disease, the IBD in the *scid* model is also characterized by transmural infiltration with deep fistulas. Unlike the other murine models of colitis, the severity of the disease is not limited to the distal colon but is of equal severity in the transverse and proximal colon.

Antibody blockade of $\beta 7$ and MAdCAM interactions

[0187] Anti-murine MAdCAM-1 antibody (MAb MECA-367; American Type Culture Collection (Rockville, MD), Accession No. HB 9478; Streeter, P.R., *et al.*, Nature, 331:41 (1988); see also, U.S. Patent No. 5,403,919 to Butcher) and anti-murine $\beta 7$ antibody (MAb FIB 504; Andrew, D.P., *et al.*, J. Immunol., 153: 3847 (1994)) were used in these studies.

[0188] *scid* mice were reconstituted with 2 X 10⁵ CD45RB^{hi} or CD45RB^{lo} CD4⁺ T cells. Five months post-reconstitution, mice were injected for 14 days with 200 μ g/day of Rat IgG2a control antibody or a mixture of 100 μ g/day FIB-504 (murine $\beta 7$ -specific) + 100 μ g/day MECA-367 (murine MAdCAM-specific). Antibody was in PBS. There were five mice in each treatment group. After 14 days, mice were injected intravenously with 5 X 10⁶ mesenteric lymph node cells (BALB/c) labeled with ¹¹¹In-oxine. 24 hours after adoptive transfer of labeled cells, tissues were harvested and assessed for radioactivity. Background levels of radioactivity in tissues, contributed by non-specific trapping of cells, were assessed by injection of 5 X 10⁶ labeled cells fixed with 2% PBS-buffered formaldehyde. Results were expressed as % counts per minute (CPM) in colon normalized to CPM in spleen and corrected for background.

[0189] This quantitative assessment of infiltration to the colon in *scid* mice reconstituted with CD45RB^{hi} CD4⁺ T cells revealed an increase in localization of 10- to 100-fold as compared with the level observed in *scid* recipients reconstituted with an equal number of CD45RB^{lo} CD4⁺ T cells. This increased accumulation of labeled cells in the colon was inhibited 50-75% by treatment for 2 weeks with a combination of anti- $\beta 7$ and anti-MAdCAM monoclonal antibodies (Figure 19).

[0190] In another experiment, *scid* mice were reconstituted with 5 X 10⁴ CD45RB^{hi} or CD45RB^{lo} CD4⁺ T cells. At the time of reconstitution, mice were treated with either (a) 500 μ g of FIB 504 ($\beta 7$ -specific) (6 mice); (b) 500 μ g MECA-367 (MAdCAM-specific) (3 mice); (c) 1 mg isotype-matched control antibody (7 mice); or (d) 1 mg FIB 504 + MECA-367 (500 μ g each) (5 mice). Following reconstitution, antibodies were administered at weekly intervals: (a) 250 μ g FIB 504; (b) 250 μ g MECA-367; (c) 500 μ g isotype-matched control; or (d) 500 μ g FIB 504 + MECA-367 (250 μ g each).

[0191] After 4 months of treatment, mice were injected with 5 X 10⁶ ¹¹¹In-labeled mesenteric lymph node cells (BALB/c), and recruitment to the colon was assessed by measuring levels of radioactivity. Results were calculated as described for Figure 19. Treatment of *scid* mice for 4 months, starting from the time of reconstitution, with FIB 504 and MECA-367, alone or in combination, inhibited the increased recruitment of lymphocytes to the colon by 100% (Figure 20).

[0192] *scid* mice were reconstituted with 2.0 X 10⁵ to 4.0 X 10⁵ CD45RB^{hi} or CD45RB^{lo} CD4⁺ T cells. After 4 months, the mice were treated for 14 days with a combination of FIB 504 ($\beta 7$ -specific) + MECA-367 (MAdCAM-specific) (100 μ g of each MAb per day for a total of 200 μ g/day) or an isotype-matched control antibody (200 μ g/day). Antibody was in

PBS. Each experimental group consisted of 4 mice. Frozen sections of left and right colon were stained with a rat monoclonal antibody specific for mouse CD4 and developed with either Fast Red or AEC (3-amino-9-ethylcarbazole) chromogen. One cross-section of left colon and right colon from each mouse was analyzed for positive staining for CD4 using a Leica Quantimet 500 Image analyzer. Each section was surveyed in its entirety using a 10X objective. Significance was determined using a students t-test. Data represents the mean positive count/tissue area $\pm$ 1 standard deviation.

[0193] Histological assessment by immunohistochemistry with a panel of antibodies specific for markers of cell lineage and state of differentiation, suggested that virtually all infiltrating cells in the colons of scid mice reconstituted with CD45RB^{hi} T cells were CD4⁺ T cells. No CD8⁺ T cells or B220⁺ B cells could be identified under the conditions used. Further, treatment of these mice with a combination of β 7- and MAdCAM-specific monoclonal antibodies significantly reduced the number of CD4⁺ T cells in the ascending or descending colon relative to the controls (Figure 21). As mesenteric lymph node cells are ~ 95% lymphocytes, these results indicate that the interaction of α 4 β 7 on lymphocytes with MAdCAM is important in the recruitment of lymphocytes to sites of inflammation in the colon and that agents which block this interaction can reduce inflammation.

Example 5. Resolution of Villus Alterations in the Common Marmoset (*Callithrix jacchus*) with Malabsorptive Enteritis

Description of model

[0194] Common marmosets (*Callithrix jacchus*) are a new world nonhuman primate that, under captive conditions at the New England Regional Primate Research Center (NERPRC), develop a steroid-nonresponsive, spontaneous malabsorption syndrome characterized by weight loss, diarrhea, and small intestinal mucosal changes consistent with loss of absorptive capacity. These histologic changes include small intestinal villus atrophy and fusion, and a mononuclear leukocyte infiltrate within the lamina propria similar to Celiac disease (nontropical sprue) in humans. Retrospective analysis from the pathology archive files at NERPRC demonstrated that up to 80% of common marmosets have, to various degrees, malabsorptive enteritis at the time of postmortem examination.

Antibody therapy protocol

[0195] Adult common marmosets were selected for study from the colony-at-large at NERPRC. Base-line studies on all animals included physical examination, complete blood count (CBC), blood chemistry profile, serum B12, c-reactive protein, and full-thickness jejunal biopsy by laparotomy. Following recovery from abdominal surgery, the animals were treated for 14 days with 2 mg/kg/day of ACT-1 monoclonal antibody, a blocking monoclonal antibody against a conformational epitope of α 4 β 7 (Schweighoffer, T., et al., J. Immunol. 151:717-729, 1993). Previous studies indicated that this antibody cross-reacted to *Callithrix* a4o7. All assessments that were performed prior to antibody therapy were repeated between the 10th and 14th day of antibody therapy.

Analysis of jejunal biopsies

[0196] Full-thickness jejunal biopsies from each marmoset were evaluated histologically by two independent pathologists, and villus architecture was scored according to the following grading criteria:

Villus atrophy

- 0 - normal mucosal thickness and villus height
- 1 - mild atrophy; slight shortening of villi; height approximately 75% of normal
- 2 - moderate atrophy; villi approximately 33-50% normal height
- 3 - severe atrophy; short (<33% normal) or no observable villi

Villus fusion

- 0 - normal; no fusion
- 1 - 1-2 villi in specimen fused
- 2 - Between 1-2 and 50% of villi in specimen fused
- 3 - >50% villi in specimen fused

Data analysis

[0197] Differences between mean scores obtained for each group of animals were assessed for statistical significance using a paired Student's t-test. Differences between means were considered significant when $P < 0.05$.

Results

[0198] The mean scores for villus fusion and atrophy before and after antibody therapy with the ACT-1 monoclonal antibody are shown in Figures 9 and 10, respectively. As demonstrated, there was almost complete resolution of villus atrophy ($P < 0.01$) and a trend for improvement of villus fusion after a two-week course of therapy with the ACT-1 antibody. The effect was not secondary to nonspecific effects of exposure to foreign immunoglobulin since other animals treated with various monoclonal antibodies directed against epitopes other than that recognized by ACT-1 were ineffective in reducing villus fusion and atrophy scores.

Example 6. Resolution of Colitis in the Cotton Top Tamarin

Description of model

[0199] The cotton-top tamarin (CTT) (*Saguinus oedipus*) is a New World nonhuman primate which develops spontaneous, and often chronic, colitis which is clinically and histologically similar to ulcerative colitis in man (Madara, J.L., et al., Gastroenterology, 88: 13-19 (1985)).

Immunotherapy. Clinical Assessment and Mucosal Biopsy

[0200] An experimental protocol involving clinical assessment, colonic mucosal biopsy, and ACT-1 immunotherapy of colitic CTTs was instituted (Figure 13). ACT-1 is a murine-IgG1 monoclonal antibody reactive with human $\alpha 4\beta 7$ (Schweighoffer, T., et al., J. Immunol., 151: 717-729 (1993); Lazarovits, A.I., et al., J. Immunol., 133: 1857-1862 (1984); and Erle, D.J., et al., J. Immunol., 153: 517-528 (1994)). ACT-1 was found to cross-react in the tamarin as assessed by immunohistologic staining with ACT-1 antibody of colitic mucosa from affected animals. These initial pilot studies demonstrated that from 40-80% of mononuclear cells within the lamina propria of colon from affected animals were $\alpha 4\beta 7+$, similar to human colitic mucosa. ACT-1 was also found to cross-react with $\alpha 4\beta 7$ from the CTT using flow cytometry on CTT peripheral blood lymphocytes (PBLs).

[0201] CTTs with chronic colitis were chosen from the colony-at-large at the New England Regional Primate Research Center, Southborough, Massachusetts based upon clinical observation of diarrhea and weight loss. To confirm the presence of colitis (as defined by a histologic inflammatory activity score of 2 or 3), colony animals noted to have clinical emaciation and diarrhea were evaluated for colonic inflammatory activity by routine histological assessment of colonic mucosal biopsy samples on multiple occasions prior to experimental assessment of antibody immunotherapy (Figure 13). Chronically colitic CTTs were screened for colitis inflammatory activity on at least two occasions by examination of mucosal specimens from the terminal descending colon, 2-3 cm from the anus, using a pediatric fiberoptic endoscope. Inflammatory activity scores were based upon the relative numbers of neutrophils within the lamina propria, crypt lumina, crypt epithelium, and surface epithelium. In particular, a histopathologic scoring system of acute and chronic inflammatory activity was used (described by Madara, J.L., et al., Gastroenterology, 88: 13-19 (1985)). All biopsy samples were scored and categorized into four groups, with 0 representing normal mucosa and 3 representing the most severe and inflamed mucosa. Scores of 0 and 1 do not represent symptomatic colitis, while scores of 2 to 3 represent mild to severe colitic activity. Animals selected for study had: (1) moderate (grade 2) or severe (grade 3) structural alterations of surface and crypt epithelium on the first biopsy specimen, suggestive of colitis of a chronic nature, and (2) moderate (grade 2) or severe (grade 3) inflammatory activity on at least two biopsy samples taken 3-7 days apart prior to immunotherapy. Biopsy samples satisfying these criteria were characterized by the presence of crypt branching and/or loss with polymorphonuclear leukocyte (PMNs) infiltration to either the lamina propria and/or epithelial compartment.

[0202] Thus, animals selected for study had repeated evidence of colonic inflammatory activity and clinically-relevant colitis of a chronic nature with no recent evidence of remission. Moreover, persistence of diarrhea to the first day of administration of monoclonal antibody was requisite for the animal to be included in the study. Within 5 days of confirmation of colitis, the animals began immunotherapy with ACT-1 monoclonal antibody.

[0203] ACT-1 antibody was produced by culture in a hollow fiber cell fermenter using a sterile pyrogen-free flowpath, purified by protein A affinity chromatography, and diluted in sterile 0.9% NaCl prior to use *in vivo*. Because CTTs are an endangered species, ACT-1 was also demonstrated to cross-react to $\alpha 4\beta 7$ on PBLs from a related species, the common marmoset (*Callithrix jacchus*) in order to perform a pharmacokinetic analysis of the antibody prior to administration to colitic CTTs. In this component of the study, ACT-1 was administered to two normal adult common marmosets,

first as a single intravenous infusion and then as a single intramuscular injection 24 hrs later. Intravenous administration of 2.0 mg/kg of ACT-1 in these animals yielded an estimated serum half life of approximately 50 hours, with continued absorption of antibody from 2-24 hours after a single intramuscular injection. Using this dosing regime, peak serum concentrations of antibody were approximately 60 µg/ml, while trough concentrations were ≥ 18.0 µg/ml. No adverse clinical effects were observed in marmosets given ACT-1.

[0204] In view of these observations, half of the cotton top tamarins satisfying the study requirements (n = 4; collective age = 31 years) were given a single intravenous (I.V.) bolus of ACT-1 at a dose of 2.0 mg/kg the first day, and 7 subsequent intramuscular (I.M.) injections of the same amount every 24 hours, for a total of 8 days of immunotherapy. The other half of the chronically colitic control animals (n = 4; collective age = 26 years) received antibody 86D, a murine monoclonal antibody (IgG1) to sheep TCR γδ (Mackay, C.R., et al., Eur. J. Immunol., 19: 1477-1483 (1989)), which does not cross-react in CTTs (data not shown). This irrelevant, isotype-matched antibody was produced, purified, and administered under identical conditions as ACT-1.

[0205] Colonic mucosal biopsies were again obtained at the time of the first antibody infusion (Day 0) and on days 5, 10 and 20. The biopsies were evaluated by an independent pathologist. Additional colon biopsies were frozen for immunohistology. For histologic analyses, colonic mucosal biopsy specimens, taken 2-3 cm for the anus, were immediately snap-frozen in OCT compound, and duplicate samples taken from the adjacent area were fixed in 10% phosphate-buffered formalin, processed by routine histological techniques, embedded in paraffin, cut at a thickness of 6.0 µm, and sections stained with hematoxylin and eosin. The formalin-fixed samples were then examined histopathologically. Acetone-fixed, frozen sections were used to detect murine IgG1 administered *in vivo* by eliminating the primary antibody in the sequence of a previously described avidin-biotin peroxidase immunohistochemical technique (Ringler, D.J., et al., Clin. Immunol. Immunopathol., 49: 349-364 (1988)).

[0206] Animal caretakers were blinded as to therapeutic regime (ACT-1 vs. isotype-matched irrelevant monoclonal antibody), and evaluated stool consistency in each animal on a daily basis by categorizing stool as diarrhea, semi-solid, or solid. Scores were assigned as follows: 0, formed, solid stool; 1, liquid stool with some solid components (semi-solid); or 2, liquid stool (diarrhea). Animals were weighed every other day, while blood was drawn at the same intervals for flow cytometry, hematology, and storage of serum or plasma for further analyses, such as antibody concentration, anti-mouse IgG titer, clinical chemistry, or acute phase proteins.

Computer-assisted morphometric image analysis

[0207] Quantitative, computer-assisted, morphometric analysis of mucosal biopsy sections was performed using a Leica Quantimet 500 Image Analyzer. First, immunohistochemical analysis of mucosal sections was performed to delineate specific leukocyte cell types using an avidin-biotin peroxidase technique, as previously described (Ringler, D.J., et al., Clin. Immunol. Immunopathol., 49: 349-364 (1988)). Paraformaldehyde-, acetone-, or formalin-fixed, frozen sections were used to identify neutrophils, β7+ cells, and monocyte/macrophages (Mφ), respectively, by using, as primary reagents in the sequence, a sheep anti-elastase polyclonal antibody (Biodesign, Kennebunk, ME) to identify neutrophils, FIB21 monoclonal antibody (rat IgG2a) to identify the β7 chain (Andrew, D.P., et al., J. Immunol., 153: 3847-3861 (1994)), and HAM-56 monoclonal antibody (mouse IgM) to identify macrophages (Dako Corp., Carpinteria, CA). Examination of stained tissue sections using the elastase antibody documented that this reagent only recognized polymorphonuclear cells in CTT colonic mucosa. Formalin-fixed, paraffin-embedded tissue sections were used to enumerate T and B cells, using a rabbit polyclonal antibody to human CD3 (Dako Corp., Carpinteria, CA) and L26 monoclonal antibody (mouse IgG2a) (Dako Corp., Carpinteria, CA), respectively, as primary reagents in the sequence. For detection of primary antibodies, species- and isotype-specific secondary reagents were used in order to eliminate recognition of ACT-1 or irrelevant murine IgG1 antibody in tissues. After immunohistochemical procedures, each cell population was enumerated on 2-4 random fields/section of mucosa. Cells were selected based on the color wavelength generated from the brown diaminobenzidine reaction product, and color selection criteria were identical on all sections analyzed for each cell-specific marker. Because of frozen section morphology and the high relative density of β7+ lymphocytes and macrophages, quantification of these cell types was evaluated as the immunoreactive fractional area of mucosa, while all other leukocyte cell types were enumerated as the cell number/mucosal area. The values were expressed as the mean (± 1 SEM) percent of the pretreatment (day 0) value within a treatment group, obtained by comparing the value from each animal's biopsy sample at a particular timepoint to the value obtained from the same animal at day 0. Thus, values less than 100% (shown in bold in Table 3 below) represent a decrease of leukocyte cell density compared to the pretreatment samples, while values greater than 100% represent an increase of mucosal leukocyte cell density. Significance was determined using a paired Student's t-test and comparing mean raw scores of cell density at a particular time point to those at pretreatment. Differences between means were considered significant when P < 0.05.

Hematology and Flow Cytometry

[0208] Lymphocytes expressing $\alpha 4\beta 7$ integrin were enumerated by flow cytometry and the ACT-1 monoclonal antibody using methods previously described (Mackay, C.R., et al., Eur. J. Immunol., 19: 1477-1483 (1989)). Because saturating serum concentrations of ACT-1 were achieved with the infusion protocol, exogenously-administered ACT-1 in the serum could be used to enumerate the number of $\alpha 4\beta 7^+$ lymphocytes in the blood. Briefly, whole blood from each animal at each blood collection was analyzed by diluting 100 μ l of EDTA-anticoagulated blood with PBS/10% rabbit serum/5% human AB serum for 20 min at 4°C. After removal of the blocking serum, in the case of animals treated with ACT-1, blood cells were directly incubated with either 100 μ l of fluorescein-conjugated, rabbit anti-mouse IgG (Dako Corporation, Carpinteria, CA), or in the case of the animals given irrelevant antibody or pretreatment blood samples, ACT-1 was added to blood at 10 μ g/ml followed then by the secondary antibody. For each sample, a minimum of 10,000 cells was analyzed. Routine blood cell counts and differential analyses were performed using a Baker 5000 hematology analyzer and appropriate gating for cotton-top tamarin red cells, white cells, and platelets. From the hematology analysis and flow cytometry results, absolute numbers of $\alpha 4\beta 7^+$ lymphocytes per μ l of blood were calculated.

Results/Progress*Serum concentrations*

[0209] Serum concentrations of ACT-1 and an irrelevant isotype-matched antibody were generally both ≥ 10 μ g/ml for the first 10 days of the study. On days 2-10 of the study, biotinylated ACT-1, used in whole blood at a concentration of 10. μ g/ml, failed to significantly label peripheral lymphocytes as assessed by flow cytometry in animals treated with ACT-1, while on day 0 and in animals treated with an irrelevant antibody, the same antibody recognized between 70-90% of the peripheral lymphocyte pool, similar to the staining profile of ACT-1 on human lymphocytes. Collectively, these results suggested that the therapeutic protocol for ACT-1 in colitic CTTs resulted in saturation of the $\alpha 4\beta 7$ integrin on lymphocytes in the peripheral circulation.

[0210] The ability of ACT-1 to recognize extravascular $\alpha 4\beta 7^+$ cells within the lamina propria of colonic mucosa of colitic CTTs was also assessed. Immunohistochemical techniques were used to detect murine IgG1 in colonic mucosal biopsies from the study animals, and ACT-1 was observed on cell membranes of mononuclear cells within the lamina propria on all biopsy time points for the first 10 days of the study in animals treated with ACT-1, but not, as expected, from Day 0 prior to antibody infusion. No labeling of lamina propria cells was observed in animals given irrelevant antibody. Therefore, the dosing regime utilized in the study resulted in neutralizing serum concentrations of ACT-1, and concomitant extravascular recognition and labeling of immune cells within colitic mucosa. ACT-1 antibody localized to the target site, namely lymphocytes within the peripheral blood and specifically to the extravascular compartment within colitic mucosa.

Clinical effect

[0211] All four test animals maintained either a grade 2 or 3 colitic inflammatory activity in both the pre-treatment and Day 0 biopsy samples, which for 3 animals was separated by 5 days. In addition, changes within the mucosal architecture of all four animals demonstrated that these four animals had colitis of a long-lasting nature. Therefore, all animals appeared to have a chronic disease course.

[0212] Histopathologic analysis of colonic mucosal biopsies was performed. The results from representative CTTs (animals Sgo 326-84 and Sgo 17-85) before and 5 days after immunotherapy with ACT-1 illustrated the therapeutic effect of ACT-1 immunotherapy on microscopic changes to the colonic mucosa in colitic CTTs. Prior to immunotherapy, there was a purulent exudate within the epithelial compartment and crypt lumen, epithelial immaturity characterized by loss of fully-differentiated goblet cells, and the lamina propria was expanded by a mononuclear and purulent inflammatory infiltrate (Sgo 326-84, muscularis mucosae). After ACT-1 immunotherapy, ACT-1 was localized to membranes of mononuclear cells within the lamina propria using immunohistochemical techniques (Sgo 17-85, muscularis mucosae), and the neutrophilic component of the inflammatory infiltrate had resolved, fully-differentiated goblet cells were observed, and the lamina propria were no longer expanded by mononuclear cells and/or neutrophils (Sgo 326-84).

[0213] The clinical effect of ACT-1 on stool consistency in colitic CTTs was striking (Figure 14). An improvement in diarrhea to at least a semi-solid stool consistency was observed in all animals within 24 hours after the first dose, while complete resolution to solid stool occurred in all animals by 72 hours. Control animals did not improve and were observed to have diarrhea for the entire study period, showing, in addition, that the preselection criteria for this group of animals effectively eliminated those with spontaneous remissions.

[0214] All animals maintained solid stools for approximately 1 week after termination of antibody injections (Figure 11). With respect to individual animals, one animal (Sgo 63-93) had solid stool from Day 4 until the end of the protocol

at Day 20 (Figure 11). Two animals (Sgo 129-91 and Sgo 17-85) had slight relapses to semi-solid stools after Day 14 in the study (Figure 11). The fourth animal (Sgo 326-84) showed a persistent improvement/ resolution of diarrhea from Day 6 to Day 20.

[0215] Similarly, leukocyte infiltrates in the colon were markedly attenuated in CTTs given ACT-1. Compared to pre-treatment biopsies, histologic analysis of colonic mucosa (formalin-fixed biopsy specimens of colonic mucosa) from animals treated with ACT-1 showed an improvement in inflammatory activity and associated structural alterations of the mucosa. Using a histologic scoring system of colonic inflammatory activity (Madara, J.L., et al., *Gastroenterology*, 88: 13-19 (1985)), animals treated with ACT-1 had marked decreases in inflammatory activity scores at all time points compared to baseline pretreatment scores, while scores from animals given the control antibody did not change (Figure 15). There were no changes in inflammatory scores for the irrelevant treatment group on Day 20 (Figure 15). Mean raw scores of inflammatory activity at all time points in the ACT-1-treated group were statistically lower than those from the same animals at Day 0 (Days 5 and 10, $P < 0.05$; Day 20, $P < 0.01$).

[0216] With respect to individual animals, all four test animals showed improvement in inflammatory activity during or after ACT-1 immunotherapy. The colitis in two animals (Sgo 129-91 and Sgo 17-85) completely resolved by Day 10 (Figure 12). Another animal (Sgo 63-93) did not show complete abrogation of colitis activity until Day 20 (Figure 12), while mucosal biopsy scores from the fourth animal (Sgo 326-84) showed improvement during the entire study period (Figure 12; two biopsies on day 20 in Sgo 326-84 were scored as 0 and 1). Furthermore, animal 326-84 gained 20% of its original body weight during the study period.

[0217] In order to provide a more quantitative assessment of efficacy, computer-assisted morphometric image analysis of leukocyte subsets within colonic mucosa from all study animals was performed. Table 3 illustrates the results of an analysis of the effect of ACT-1 immunotherapy on colonic inflammatory activity in chronically colitic CTTs (expressed as a percent of pretreatment values). ACT-1 immunotherapy resulted in significant reductions in the densities of mucosal leukocytes compared to baseline numbers established prior to antibody administration, while the control group generally had either similar or increased numbers of mucosal leukocytes after administration of irrelevant antibody. Ten days after the first dose of ACT-1 antibody, there were approximately 30% fewer mucosal mononuclear leukocytes expressing $\beta 7$ integrins compared to pretreatment values. This decrease was not attributed to decreases in the numbers of $\alpha 4\beta 7$ lymphocytes in the peripheral circulatory pool (Figure 16), nor was it related to manipulation or nonspecific effects since the numbers of $\beta 7$ leukocytes in colonic mucosa from the control animals either increased or remained largely unchanged. Similarly, mucosal T cells were reduced by approximately 50% at day 5 and by about 25% by day 10 in animals treated with ACT-1, while mucosal T cells in the irrelevant antibody group at the same time points did not change. Comparable reductions in mucosal B cells were seen in the ACT-1-treated group but not in the control group. Interestingly, ACT-1 immunotherapy also reduced the density of mucosal leukocytes which have either little or no expression of $\alpha 4\beta 7$. Neutrophils were reduced by 40-45% at days 10 and 20 in animals treated with ACT-1, yet reductions in PMNs were not observed in the control group. Similarly, mucosal macrophages were reduced in all post-treatment biopsy samples by 30-45% in animals given ACT-1, while macrophages in the control group did not change. Interestingly, using immunohistochemistry and a cross-reactive monoclonal antibody specific for cloned human MAdCAM (10G3, Example 2), no change in expression was observed in colitic CTTs treated with ACT-1 or control antibody.

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TABLE 3

Quantitative analysis of leukocyte density in colonic mucosa of CTTs treated with ACT-1 or control monoclonal antibodies										
ACT-1						Irrelevant IgG1				
	PMNs	β7 ⁺ Cells	T Cells	B Cells	Mφ	PMNs	β7 ⁺ Cells	T Cells	B Cells	Mφ
Day 5 (%)	83.0±11.3	69.9±12.0	50.4±5.9 ¶¶	53.1±11.8†	70.3±6.4‡	75.7±17.8	73.8±10.0	104.0±8.0	256.1±67.7	141.8±21.6
Day 10 (%)	57.5±13.7†	68.4±7.1†	75.9±44.4§	82.1±17.6	58.5±4.3¶¶	148.3±49.3	118.8±27.6	105.6±9.6	357.0±108.4	80.7±9.8
Day 20 (%)	61.1±11.4§	59.8±12.6§	96.4±10.4	61.3± 13.4§	58.5±5.0§	339.6±94.1	113.7±28.1	72.4±11.3†	209.0±51.0	174.5±48.0
†P < 0.05; ‡P < 0.02; § P < 0.01; ¶¶ P < 0.001.										

[0218] No overt toxicity was observed in the study animals that could be attributed to ACT-1 administration. None of the study animals showed changes in clinical chemistry assessments of liver and renal function (data not shown). The ACT-1 antibody is a nonlytic monoclonal reagent (Lazarovits, A.I., et al., J. Immunol., 133: 1857-1862 (1984)), and leukopenia was not observed in any animal during the study. Indeed, there was a trend for neutrophilia (peak numbers in peripheral blood approaching $40 \times 10^3/\mu\text{l}$; CTT normal range is $1.4 - 12.0 \times 10^3/\mu\text{l}$) in all study animals, including the control animals, during the first week of study when daily anesthesia/manipulation was used to administer the antibodies. There was also a trend for lymphocytosis in the animals given ACT-1, with absolute numbers of lymphocytes in peripheral blood approaching $18 \times 10^3/\mu\text{l}$ (CTT normal range is $0.6 - 5.7 \times 10^3/\mu\text{l}$). Therefore, decreased recruitment of any leukocyte cell type to the colon in the ACT-1-treated group could not be attributed to changes in the number of leukocytes in the peripheral circulatory pool.

Summary

[0219] When administered to chronically colitic cotton-top tamarins, a monoclonal antibody to $\alpha 4\beta 7$ integrin rapidly resolved diarrhea and colonic inflammatory activity, indicating efficacy in improving colitis. There appeared to be a good correlation between histologic inflammatory activity scores and stool consistency. The observation that stool consistency generally improved in 1-2 days in animals receiving ACT-1 antibody is noteworthy. Furthermore, the relative density of mucosal leukocyte subsets was greatly attenuated in response to immunotherapy with anti- $\alpha 4\beta 7$ antibodies. These results also demonstrate an efficacious therapy for an inflammatory process which may be organ- or tissue specific (mucosal-specific).

[0220] The therapeutic effect of ACT-1 in colitic CTTs may be mediated by inhibition of lymphocyte recruitment to gut. Alternatively, or in addition, the therapeutic effect observed may reflect alterations in other cell interactions or signalling events mediated by $\alpha 4\beta 7$ integrin. These results indicate that ACT-1 antibody is an effective antagonist of $\alpha 4\beta 7$ integrin function, and that inhibition of $\alpha 4\beta 7$ integrin function can be an organ- or tissue-specific treatment modality in the clinical management of individuals with inflammatory bowel disease. Further, the results indicate a role for $\alpha 4\beta 7$ integrin in the pathogenesis of inflammatory bowel disease. $\alpha 4\beta 7$ integrin provides a potentially organ-specific, therapeutic target for inflammatory bowel disease.

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(v) COMPUTER READABLE FORM:

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40

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(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 1624 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..1218

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG GAT TTC GGA CTG GCC CTC CTG CTG GCG GGG CTT CTG GGG CTC CTC	48
Met Asp Phe Gly Leu Ala Leu Leu Leu Ala Gly Leu Leu Gly Leu Leu	
1 5 10 15	
CTC GGC CAG TCC CTC CAG GTG AAG CCC CTG CAG GTG GAG CCC CCG GAG	96
Leu Gly Gln Ser Leu Gln Val Lys Pro Leu Gln Val Glu Pro Pro Glu	
20 25 30	
CCG GTG GTG GCC GTG GCC TTG GGC GCC TCG CGC CAG CTC ACC TGC CGC	144
Pro Val Val Ala Val Ala Leu Gly Ala Ser Arg Gln Leu Thr Cys Arg	
35 40 45	

	CTG	GCC	TGC	GCG	GAC	CGC	GGG	GCC	TCG	GTG	CAG	TGG	CGG	GGC	CTG	GAC	192
	Leu	Ala	Cys	Ala	Asp	Arg	Gly	Ala	Ser	Val	Gln	Trp	Arg	Gly	Leu	Asp	
		50					55					60					
5	ACC	AGC	CTG	GGC	GCG	GTG	CAG	TCG	GAC	ACG	GGC	CGC	AGC	GTC	CTC	ACC	240
	Thr	Ser	Leu	Gly	Ala	Val	Gln	Ser	Asp	Thr	Gly	Arg	Ser	Val	Leu	Thr	
	65					70					75					80	
10	GTG	CGC	AAC	GCC	TCG	CTG	TCG	GCG	GCC	GGG	ACC	CGC	GTG	TGC	GTG	GGC	288
	Val	Arg	Asn	Ala	Ser	Leu	Ser	Ala	Ala	Gly	Thr	Arg	Val	Cys	Val	Gly	
					85					90					95		
	TCC	TGC	GGG	GGC	CGC	ACC	TTC	CAG	CAC	ACC	GTG	CAG	CTC	CTT	GTG	TAC	336
	Ser	Cys	Gly	Gly	Arg	Thr	Phe	Gln	His	Thr	Val	Gln	Leu	Leu	Val	Tyr	
				100					105					110			
15	GCC	TTC	CCG	GAC	CAG	CTG	ACC	GTC	TCC	CCA	GCA	GCC	CTG	GTG	CCT	GGT	384
	Ala	Phe	Pro	Asp	Gln	Leu	Thr	Val	Ser	Pro	Ala	Ala	Leu	Val	Pro	Gly	
			115					120					125				
20	GAC	CCG	GAG	GTG	GCC	TGT	ACG	GCC	CAC	AAA	GTC	ACG	CCC	GTG	GAC	CCC	432
	Asp	Pro	Glu	Val	Ala	Cys	Thr	Ala	His	Lys	Val	Thr	Pro	Val	Asp	Pro	
		130					135					140					
	AAC	GCG	CTC	TCC	TTC	TCC	CTG	CTC	GTC	GGG	GGC	CAG	GAA	CTG	GAG	GGG	480
	Asn	Ala	Leu	Ser	Phe	Ser	Leu	Leu	Val	Gly	Gly	Gln	Glu	Leu	Glu	Gly	
		145				150					155					160	
25	GCG	CAA	GCC	CTG	GGC	CCG	GAG	GTG	CAG	GAG	GAG	GAG	GAG	GAG	CCC	CAG	528
	Ala	Gln	Ala	Leu	Gly	Pro	Glu	Val	Gln	Glu	Glu	Glu	Glu	Glu	Pro	Gln	
					165					170					175		
	GGG	GAC	GAG	GAC	GTG	CTG	TTC	AGG	GTG	ACA	GAG	CGC	TGG	CGG	CTG	CCG	576
	Gly	Asp	Glu	Asp	Val	Leu	Phe	Arg	Val	Thr	Glu	Arg	Trp	Arg	Leu	Pro	
				180					185					190			
30	CCC	CTG	GGG	ACC	CCT	GTC	CCG	CCC	GCC	CTC	TAC	TGC	CAG	GCC	ACG	ATG	624
	Pro	Leu	Gly	Thr	Pro	Val	Pro	Pro	Ala	Leu	Tyr	Cys	Gln	Ala	Thr	Met	
			195				200						205				
	AGG	CTG	CCT	GGC	TTG	GAG	CTC	AGC	CAC	CGC	CAG	GCC	ATC	CCC	GTC	CTG	672
	Arg	Leu	Pro	Gly	Leu	Glu	Leu	Ser	His	Arg	Gln	Ala	Ile	Pro	Val	Leu	
		210				215						220					
35	CAC	AGC	CCG	ACC	TCC	CCG	GAG	CCT	CCC	GAC	ACC	ACC	TCC	CCG	GAG	CCT	720
	His	Ser	Pro	Thr	Ser	Pro	Glu	Pro	Pro	Asp	Thr	Thr	Ser	Pro	Glu	Pro	
						230					235					240	
40	CCC	AAC	ACC	ACC	TCC	CCG	GAG	TCT	CCC	GAC	ACC	ACC	TCC	CCG	GAG	TCT	768
	Pro	Asn	Thr	Thr	Ser	Pro	Glu	Ser	Pro	Asp	Thr	Thr	Ser	Pro	Glu	Ser	
					245					250					255		
	CCC	GAC	ACC	ACC	TCC	CAG	GAG	CCT	CCC	GAC	ACC	ACC	TCC	CAG	GAG	CCT	816
	Pro	Asp	Thr	Thr	Ser	Gln	Glu	Pro	Pro	Asp	Thr	Thr	Ser	Gln	Glu	Pro	
					260				265					270			
45	CCC	GAC	ACC	ACC	TCC	CAG	GAG	CCT	CCC	GAC	ACC	ACC	TCC	CCG	GAG	CCT	864
	Pro	Asp	Thr	Thr	Ser	Gln	Glu	Pro	Pro	Asp	Thr	Thr	Ser	Pro	Glu	Pro	
			275					280					285				
50																	
55																	

	CCC GAC AAG ACC TCC CCG GAG CCC GCC CCC CAG CAG GGC TCC ACA CAC	912
	Pro Asp Lys Thr Ser Pro Glu Pro Ala Pro Gln Gln Gly Ser Thr His	
	290 295 300	
5	ACC CCC AGG AGC CCA GGC TCC ACC AGG ACT CGC CGC CCT GAG ATC TCC	960
	Thr Pro Arg Ser Pro Gly Ser Thr Arg Thr Arg Arg Pro Glu Ile Ser	
	305 310 315 320	
10	CAG GCT GGG CCC ACG CAG GGA GAA GTG ATC CCA ACA GGC TCG TCC AAA	1008
	Gln Ala Gly Pro Thr Gln Gly Glu Val Ile Pro Thr Gly Ser Ser Lys	
	325 330 335	
	CCT GCG GGT GAC CAG CTG CCC GCG GCT CTG TGG ACC AGC AGT GCG GTG	1056
	Pro Ala Gly Asp Gln Leu Pro Ala Ala Leu Trp Thr Ser Ser Ala Val	
	340 345 350	
15	CTG GGA CTG CTG CTC CTG GCC TTG CCC ACG TAT CAC CTC TGG AAA CGC	1104
	Leu Gly Leu Leu Leu Leu Ala Leu Pro Thr Tyr His Leu Trp Lys Arg	
	355 360 365	
20	TGC CGG CAC CTG GCT GAG GAC GAC ACC CAC CCA CCA GCT TCT CTG AGG	1152
	Cys Arg His Leu Ala Glu Asp Asp Thr His Pro Pro Ala Ser Leu Arg	
	370 375 380	
	CTT CTG CCC CAG GTG TCG GCC TGG GCT GGG TTA AGG GGG ACC GGC CAG	1200
	Leu Leu Pro Gln Val Ser Ala Trp Ala Gly Leu Arg Gly Thr Gly Gln	
	385 390 395 400	
25	GTC GGG ATC AGC CCC TCC TGAGTGGCCA GCCTTTCCCC CTGTGAAAGC	1248
	Val Gly Ile Ser Pro Ser	
	405	
	AAAATAGCTT GGACCCCTTC AAGTTGAGAA CTGGTCAGGG CAAACCTGCC TCCCATTCTA	1308
	CTCAAAGTCA TCCCTCTGCT CACAGAGATG GATGCATGTT CTGATTGCCT CTTTGGAGAA	1368
30	GCTCATCAGA AACTCAAAAG AAGGCCACTG TTTGTCTCAC CTACCCATGA CCTGAAGCCC	1428
	CTCCCTGAGT GGTCCCCACC TTTCTGGACG GAACCACGTA CTTTTTACAT ACATTGATTC	1488
	ATGTCTCACG TCTCCCTAAA AATGCGTAAG ACCAAGCTGT GCCCTGACCA CCCTGGGCCC	1548
35	CTGTGCTCAG GACCTCCTGA GGCTTTGGCA AATAAACCTC CTAAAATGAT AAAAAAAAAA	1608
	AAAAAAAAAA AAAAAA	1624

40 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 406 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asp Phe Gly Leu Ala Leu Leu Leu Ala Gly Leu Leu Gly Leu Leu
 1 5 10 15

55

Leu Gly Gln Ser Leu Gln Val Lys Pro Leu Gln Val Glu Pro Pro Glu
 20 25 30
 5 Pro Val Val Ala Val Ala Leu Gly Ala Ser Arg Gln Leu Thr Cys Arg
 35 40 45
 Leu Ala Cys Ala Asp Arg Gly Ala Ser Val Gln Trp Arg Gly Leu Asp
 50 55 60
 10 Thr Ser Leu Gly Ala Val Gln Ser Asp Thr Gly Arg Ser Val Leu Thr
 65 70 75 80
 Val Arg Asn Ala Ser Leu Ser Ala Ala Gly Thr Arg Val Cys Val Gly
 85 90 95
 15 Ser Cys Gly Gly Arg Thr Phe Gln His Thr Val Gln Leu Leu Val Tyr
 100 105 110
 Ala Phe Pro Asp Gln Leu Thr Val Ser Pro Ala Ala Leu Val Pro Gly
 115 120 125
 20 Asp Pro Glu Val Ala Cys Thr Ala His Lys Val Thr Pro Val Asp Pro
 130 135 140
 Asn Ala Leu Ser Phe Ser Leu Leu Val Gly Gly Gln Glu Leu Glu Gly
 145 150 155 160
 25 Ala Gln Ala Leu Gly Pro Glu Val Gln Glu Glu Glu Glu Glu Pro Gln
 165 170 175
 Gly Asp Glu Asp Val Leu Phe Arg Val Thr Glu Arg Trp Arg Leu Pro
 180 185 190
 30 Pro Leu Gly Thr Pro Val Pro Pro Ala Leu Tyr Cys Gln Ala Thr Met
 195 200 205
 Arg Leu Pro Gly Leu Glu Leu Ser His Arg Gln Ala Ile Pro Val Leu
 210 215 220
 35 His Ser Pro Thr Ser Pro Glu Pro Pro Asp Thr Thr Ser Pro Glu Pro
 225 230 235 240
 Pro Asn Thr Thr Ser Pro Glu Ser Pro Asp Thr Thr Ser Pro Glu Ser
 245 250 255
 40 Pro Asp Thr Thr Ser Gln Glu Pro Pro Asp Thr Thr Ser Gln Glu Pro
 260 265 270
 Pro Asp Thr Thr Ser Gln Glu Pro Pro Asp Thr Thr Ser Pro Glu Pro
 275 280 285
 45 Pro Asp Lys Thr Ser Pro Glu Pro Ala Pro Gln Gln Gly Ser Thr His
 290 295 300
 Thr Pro Arg Ser Pro Gly Ser Thr Arg Thr Arg Arg Pro Glu Ile Ser
 305 310 315 320
 50 Gln Ala Gly Pro Thr Gln Gly Glu Val Ile Pro Thr Gly Ser Ser Lys
 325 330 335
 Pro Ala Gly Asp Gln Leu Pro Ala Ala Leu Trp Thr Ser Ser Ala Val
 340 345 350
 55

Leu Gly Leu Leu Leu Leu Ala Leu Pro Thr Tyr His Leu Trp Lys Arg
 355 360 365

Cys Arg His Leu Ala Glu Asp Asp Thr His Pro Pro Ala Ser Leu Arg
 370 375 380

Leu Leu Pro Gln Val Ser Ala Trp Ala Gly Leu Arg Gly Thr Gly Gln
 385 390 395 400

Val Gly Ile Ser Pro Ser
 405

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1539 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1146

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG GAT TTC GGA CTG GCC CTC CTG CTG GCG GGG CTT CTG GGG CTC CTC	48
Met Asp Phe Gly Leu Ala Leu Leu Leu Ala Gly Leu Leu Gly Leu Leu	
1 5 10 15	
CTC GGC CAG TCC CTC CAG GTG AAG CCC CTG CAG GTG GAG CCC CCG GAG	96
Leu Gly Gln Ser Leu Gln Val Lys Pro Leu Gln Val Glu Pro Pro Glu	
20 25 30	
CCG GTG GTG GCC GTG GCC TTG GGC GCC TCG CGC CAG CTC ACC TGC CGC	144
Pro Val Val Ala Val Ala Leu Gly Ala Ser Arg Gln Leu Thr Cys Arg	
35 40 45	
CTG GCC TGC GCG GAC CGC GGG GCC TCG GTG CAG TGG CGG GGC CTG GAC	192
Leu Ala Cys Ala Asp Arg Gly Ala Ser Val Gln Trp Arg Gly Leu Asp	
50 55 60	
ACC AGC CTG GGC GCG GTG CAG TCG GAC ACG GGC CGC AGC GTC CTC ACC	240
Thr Ser Leu Gly Ala Val Gln Ser Asp Thr Gly Arg Ser Val Leu Thr	
65 70 75 80	
GTG CGC AAC GCC TCG CTG TCG GCG GCC GGG ACC CGC GTG TGC GTG GGC	288
Val Arg Asn Ala Ser Leu Ser Ala Ala Gly Thr Arg Val Cys Val Gly	
85 90 95	
TCC TGC GGG GGC CGC ACC TTC CAG CAC ACC GTG CAG CTC CTT GTG TAC	336
Ser Cys Gly Gly Arg Thr Phe Gln His Thr Val Gln Leu Leu Val Tyr	
100 105 110	

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5	GCC	TTC	CCG	GAC	CAG	CTG	ACC	GTC	TCC	CCA	GCA	GCC	CTG	GTG	CCT	GGT	384
	Ala	Phe	Pro	Asp	Gln	Leu	Thr	Val	Ser	Pro	Ala	Ala	Leu	Val	Pro	Gly	
			115					120					125				
10	GAC	CCG	GAG	GTG	GCC	TGT	ACG	GCC	CAC	AAA	GTC	ACG	CCC	GTG	GAC	CCC	432
	Asp	Pro	Glu	Val	Ala	Cys	Thr	Ala	His	Lys	Val	Thr	Pro	Val	Asp	Pro	
		130					135					140					
15	AAC	GCG	CTC	TCC	TTC	TCC	CTG	CTC	GTC	GGG	GGC	CAG	GAA	CTG	GAG	GGG	480
	Asn	Ala	Leu	Ser	Phe	Ser	Leu	Leu	Val	Gly	Gly	Gln	Glu	Leu	Glu	Gly	
	145					150					155					160	
20	GCG	CAA	GCC	CTG	GGC	CCG	GAG	GTG	CAG	GAG	GAG	GAG	GAG	GAG	CCC	CAG	528
	Ala	Gln	Ala	Leu	Gly	Pro	Glu	Val	Gln	Glu	Glu	Glu	Glu	Glu	Pro	Gln	
					165					170					175		
25	GGG	GAC	GAG	GAC	GTG	CTG	TTC	AGG	GTG	ACA	GAG	CGC	TGG	CGG	CTG	CCG	576
	Gly	Asp	Glu	Asp	Val	Leu	Phe	Arg	Val	Thr	Glu	Arg	Trp	Arg	Leu	Pro	
				180					185					190			
30	CCC	CTG	GGG	ACC	CCT	GTC	CCG	CCC	GCC	CTC	TAC	TGC	CAG	GCC	ACG	ATG	624
	Pro	Leu	Gly	Thr	Pro	Val	Pro	Pro	Ala	Leu	Tyr	Cys	Gln	Ala	Thr	Met	
			195					200					205				
35	AGG	CTG	CCT	GGC	TTG	GAG	CTC	AGC	CAC	CGC	CAG	GCC	ATC	CCC	GTC	CTG	672
	Arg	Leu	Pro	Gly	Leu	Glu	Leu	Ser	His	Arg	Gln	Ala	Ile	Pro	Val	Leu	
		210					215					220					
40	CAC	AGC	CCG	ACC	TCC	CCG	GAG	CCT	CCC	GAC	ACC	ACC	TCC	CCG	GAG	TCT	720
	His	Ser	Pro	Thr	Ser	Pro	Glu	Pro	Pro	Asp	Thr	Thr	Ser	Pro	Glu	Ser	
	225					230					235					240	
45	CCC	GAC	ACC	ACC	TCC	CCG	GAG	TCT	CCC	GAC	ACC	ACC	TCC	CAG	GAG	CCT	768
	Pro	Asp	Thr	Thr	Ser	Pro	Glu	Ser	Pro	Asp	Thr	Thr	Ser	Gln	Glu	Pro	
					245					250					255		
50	CCC	GAC	ACC	ACC	TCC	CCG	GAG	CCT	CCC	GAC	AAG	ACC	TCC	CCG	GAG	CCC	816
	Pro	Asp	Thr	Thr	Ser	Pro	Glu	Pro	Pro	Asp	Lys	Thr	Ser	Pro	Glu	Pro	
					260				265					270			
55	GCC	CCC	CAG	CAG	GGC	TCC	ACA	CAC	ACC	CCC	AGG	AGC	CCA	GGC	TCC	ACC	864
	Ala	Pro	Gln	Gln	Gly	Ser	Thr	His	Thr	Pro	Arg	Ser	Pro	Gly	Ser	Thr	
			275					280					285				
60	AGG	ACT	CGC	CGC	CCT	GAG	ATC	TCC	CAG	GCT	GGG	CCC	ACG	CAG	GGA	GAA	912
	Arg	Thr	Arg	Arg	Pro	Glu	Ile	Ser	Gln	Ala	Gly	Pro	Thr	Gln	Gly	Glu	
		290					295					300					
65	GTG	ATC	CCA	ACA	GGC	TCG	TCC	AAA	CCT	GCG	GGT	GAC	CAG	CTG	CCC	GCG	960
	Val	Ile	Pro	Thr	Gly	Ser	Ser	Lys	Pro	Ala	Gly	Asp	Gln	Leu	Pro	Ala	
	305					310					315					320	
70	GCT	CTG	TGG	ACC	AGC	AGT	GCG	GTG	CTG	GGA	CTG	CTG	CTC	CTG	GCC	TTG	1008
	Ala	Leu	Trp	Thr	Ser	Ser	Ala	Val	Leu	Gly	Leu	Leu	Leu	Leu	Ala	Leu	
					325					330					335		
75	CCC	ACC	TAT	CAC	CTC	TGG	AAA	CGC	TGC	CGG	CAC	CTG	GCT	GAG	GAC	GAC	1056
	Pro	Thr	Tyr	His	Leu	Trp	Lys	Arg	Cys	Arg	His	Leu	Ala	Glu	Asp	Asp	
				340					345					350			

ACC CAC CCA CCA GCT TCT CTG AGG CTT CTG CCC CAG GTG TCG GCC TGG 1104
 Thr His Pro Pro Ala Ser Leu Arg Leu Leu Pro Gln Val Ser Ala Trp
 355 360 365
 5 GCT GGG TTA AGG GGG ACC GGC CAG GTC GGG ATC AGC CCC TCC 1146
 Ala Gly Leu Arg Gly Thr Gly Gln Val Gly Ile Ser Pro Ser
 370 375 380
 TGAGTGGCCA GCCTTTCCCC CTGTGAAAGC AAAATAGCTT GGACCCCTTC AAGTTGAGAA 1206
 10 CTGGTCAGGG CAAACCTGCC TCCCATTCTA CTCAAAGTCA TCCCTCTGTT CACAGAGATG 1266
 GATGCATGTT CTGATTGCCT CTTTGGAGAA GCTCATCAGA AACTCAAAAG AAGGCCACTG 1326
 TTTGTCTCAC CTACCCATGA CCTGAAGCCC CTCCTGAGT GGTCCCCACC TTTCTGGACG 1386
 15 GAACCACGTA CTTTTTACAT ACATTGATTC ATGTCTCACG TCTCCCTAAA AATGCGTAAG 1446
 ACCAAGCTGT GCCCTGACCA CCCTGGGCCC CTGTCGTCAG GACCTCCTGA GGCTTTGGCA 1506
 AATAAACCTC CTAAATGAA AAAAAAAAAA AAA 1539
 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 382 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

35 Met Asp Phe Gly Leu Ala Leu Leu Leu Ala Gly Leu Leu Gly Leu Leu
 1 5 10 15
 Leu Gly Gln Ser Leu Gln Val Lys Pro Leu Gln Val Glu Pro Pro Glu
 20 25 30
 40 Pro Val Val Ala Val Ala Leu Gly Ala Ser Arg Gln Leu Thr Cys Arg
 35 40 45
 Leu Ala Cys Ala Asp Arg Gly Ala Ser Val Gln Trp Arg Gly Leu Asp
 50 55 60
 45 Thr Ser Leu Gly Ala Val Gln Ser Asp Thr Gly Arg Ser Val Leu Thr
 65 70 75 80
 Val Arg Asn Ala Ser Leu Ser Ala Ala Gly Thr Arg Val Cys Val Gly
 85 90 95
 50 Ser Cys Gly Gly Arg Thr Phe Gln His Thr Val Gln Leu Leu Val Tyr
 100 105 110
 Ala Phe Pro Asp Gln Leu Thr Val Ser Pro Ala Ala Leu Val Pro Gly
 115 120 125
 55

Asp Pro Glu Val Ala Cys Thr Ala His Lys Val Thr Pro Val Asp Pro
 130 135 140
 5 Asn Ala Leu Ser Phe Ser Leu Leu Val Gly Gly Gln Glu Leu Glu Gly
 145 150 155 160
 Ala Gln Ala Leu Gly Pro Glu Val Gln Glu Glu Glu Glu Glu Pro Gln
 165 170 175
 10 Gly Asp Glu Asp Val Leu Phe Arg Val Thr Glu Arg Trp Arg Leu Pro
 180 185 190
 Pro Leu Gly Thr Pro Val Pro Pro Ala Leu Tyr Cys Gln Ala Thr Met
 195 200 205
 15 Arg Leu Pro Gly Leu Glu Leu Ser His Arg Gln Ala Ile Pro Val Leu
 210 215 220
 His Ser Pro Thr Ser Pro Glu Pro Pro Asp Thr Thr Ser Pro Glu Ser
 225 230 235 240
 20 Pro Asp Thr Thr Ser Pro Glu Ser Pro Asp Thr Thr Ser Gln Glu Pro
 245 250 255
 Pro Asp Thr Thr Ser Pro Glu Pro Pro Asp Lys Thr Ser Pro Glu Pro
 260 265 270
 25 Ala Pro Gln Gln Gly Ser Thr His Thr Pro Arg Ser Pro Gly Ser Thr
 275 280 285
 Arg Thr Arg Arg Pro Glu Ile Ser Gln Ala Gly Pro Thr Gln Gly Glu
 290 295 300
 30 Val Ile Pro Thr Gly Ser Ser Lys Pro Ala Gly Asp Gln Leu Pro Ala
 305 310 315 320
 Ala Leu Trp Thr Ser Ser Ala Val Leu Gly Leu Leu Leu Leu Ala Leu
 325 330 335
 35 Pro Thr Tyr His Leu Trp Lys Arg Cys Arg His Leu Ala Glu Asp Asp
 340 345 350
 Thr His Pro Pro Ala Ser Leu Arg Leu Leu Pro Gln Val Ser Ala Trp
 355 360 365
 40 Ala Gly Leu Arg Gly Thr Gly Gln Val Gly Ile Ser Pro Ser
 370 375 380

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1721 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 4..1038

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

5	AGC	ATG	GAT	CGG	GGC	CTG	GCC	CTC	CTG	CTG	GCG	GGG	CTT	CTG	GGG	CTC	48
	Met	Asp	Arg	Gly	Leu	Ala	Leu	Leu	Leu	Ala	Gly	Leu	Leu	Gly	Leu		
	1				5					10					15		
	CTC	CAG	CCG	GGC	TGC	GGC	CAG	TCC	CTC	CAG	GTG	AAG	CCC	CTG	CAG	GTG	96
	Leu	Gln	Pro	Gly	Cys	Gly	Gln	Ser	Leu	Gln	Val	Lys	Pro	Leu	Gln	Val	
				20						25					30		
10	GAG	CCC	CCG	GAG	CCG	GTG	GTG	GCC	GTG	GCC	CTG	GGC	GCC	TCT	CGC	CAG	144
	Glu	Pro	Pro	Glu	Pro	Val	Val	Ala	Val	Ala	Leu	Gly	Ala	Ser	Arg	Gln	
				35					40					45			
15	CTC	ACC	TGC	CGC	CTG	GAC	TGC	GCG	GAC	CGC	GGG	GCC	ACG	GTG	CAG	TGG	192
	Leu	Thr	Cys	Arg	Leu	Asp	Cys	Ala	Asp	Arg	Gly	Ala	Thr	Val	Gln	Trp	
			50					55					60				
	CGG	GGC	CTG	GAC	ACC	AGC	CTG	GGC	GCG	GTG	CAG	TCG	GAC	GCG	GGC	CGC	240
	Arg	Gly	Leu	Asp	Thr	Ser	Leu	Gly	Ala	Val	Gln	Ser	Asp	Ala	Gly	Arg	
		65					70					75					
20	AGC	GTC	CTC	ACC	GTG	CGC	AAC	GCC	TCG	CTG	TCG	GCG	GCC	GGG	ACC	CGT	288
	Ser	Val	Leu	Thr	Val	Arg	Asn	Ala	Ser	Leu	Ser	Ala	Ala	Gly	Thr	Arg	
		80				85					90					95	
25	GTG	TGC	GTG	GGC	TCC	TGC	GGG	GGC	CGC	ACC	TTC	CAG	CAC	ACC	GTG	CGG	336
	Val	Cys	Val	Gly	Ser	Cys	Gly	Gly	Arg	Thr	Phe	Gln	His	Thr	Val	Arg	
					100					105					110		
	CTC	CTT	GTG	TAC	GCC	TTC	CCG	GAC	CAG	CTG	ACC	ATC	TCC	CCG	GCA	GCC	384
	Leu	Leu	Val	Tyr	Ala	Phe	Pro	Asp	Gln	Leu	Thr	Ile	Ser	Pro	Ala	Ala	
				115					120					125			
30	CTG	GTG	CCT	GGT	GAC	CCG	GAG	GTG	GCC	TGT	ACG	GCC	CAC	AAA	GTC	ACG	432
	Leu	Val	Pro	Gly	Asp	Pro	Glu	Val	Ala	Cys	Thr	Ala	His	Lys	Val	Thr	
			130					135					140				
35	CCT	GTG	GAC	CCC	AAT	GCG	CTC	TCC	TTC	TCC	CTG	CTC	CTG	GGG	GAC	CAG	480
	Pro	Val	Asp	Pro	Asn	Ala	Leu	Ser	Phe	Ser	Leu	Leu	Leu	Gly	Asp	Gln	
		145					150					155					
	GAA	CTG	GAG	GGG	GCC	CAG	GCT	CTG	GGC	CCG	GAG	GTG	GAG	GAG	GAG	GAG	528
	Glu	Leu	Glu	Gly	Ala	Gln	Ala	Leu	Gly	Pro	Glu	Val	Glu	Glu	Glu	Glu	
		160				165					170					175	
40	GAG	GAG	CCC	CAG	GAG	GAG	GAG	GAC	GTG	CTG	TTC	AGG	GTG	ACA	GAG	CGC	576
	Glu	Glu	Pro	Gln	Glu	Glu	Glu	Asp	Val	Leu	Phe	Arg	Val	Thr	Glu	Arg	
					180					185					190		
45	TGG	CGG	CTG	CCG	ACC	CTG	GCA	ACC	CCT	GTC	CTG	CCC	GCG	CTC	TAC	TGC	624
	Trp	Arg	Leu	Pro	Thr	Leu	Ala	Thr	Pro	Val	Leu	Pro	Ala	Leu	Tyr	Cys	
				195					200					205			
50																	
55																	

	CAG GCC ACG ATG AGG CTG CCT GGC TTG GAG CTC AGC CAC CGC CAG GCC	672
	Gln Ala Thr Met Arg Leu Pro Gly Leu Glu Leu Ser His Arg Gln Ala	
	210 215 220	
5	ATC CCG GTC CTG CAC GGC CCG ACC TCC CGG GAG CCC CCC GAC ACG ACC	720
	Ile Pro Val Leu His Gly Pro Thr Ser Arg Glu Pro Pro Asp Thr Thr	
	225 230 235	
10	TCC CCG GAA CCC CGG GCC GCG ACC TCC CCG GAG ACC ACC CCC CAG CAG	768
	Ser Pro Glu Pro Arg Ala Ala Thr Ser Pro Glu Thr Thr Pro Gln Gln	
	240 245 250 255	
	GGC TCC ACA CAC AGC CCC AGG AGC CCG GGC TCT ACC AGG ACT TGC CGC	816
	Gly Ser Thr His Ser Pro Arg Ser Pro Gly Ser Thr Arg Thr Cys Arg	
	260 265 270	
15	CCT GAG ATC TCC CAG GCT GGG CCC ACG CAG GGA GAA GTG ATC CCA ACA	864
	Pro Glu Ile Ser Gln Ala Gly Pro Thr Gln Gly Glu Val Ile Pro Thr	
	275 280 285	
20	GGC TCG TCC AAA CCT ACG GGT GAC CAG CTG CCC GCG GCT CTG TGG ACC	912
	Gly Ser Ser Lys Pro Thr Gly Asp Gln Leu Pro Ala Ala Leu Trp Thr	
	290 295 300	
	AGC AGT GCG GTG CTG GGA CTG CTG CTC CTG GCT TTG CCC ACC TAC CAC	960
	Ser Ser Ala Val Leu Gly Leu Leu Leu Leu Ala Leu Pro Thr Tyr His	
	305 310 315	
25	CTC TGG AAA CGT TGC CGG CAC CTG GCT GAG GAC GGC GCC CAC CCA CCA	1008
	Leu Trp Lys Arg Cys Arg His Leu Ala Glu Asp Gly Ala His Pro Pro	
	320 325 330 335	
30	GCT TCT CTG AGT AGC CAG CCC TTC CCC CTG TGAAGGGAAA ATAGGTTGGA	1058
	Ala Ser Leu Ser Ser Gln Pro Phe Pro Leu	
	340 345	
	CCCCTTCAAG CTGAGAACTG GTCGGGGCAA ACCTGCCTCC CATTCTATTC AAAGTCATCG	1118
	CTCTGGTCAC AGAGAGGGAC GCACATTCTG ATTGCCTCCT TTGGAAAGGC TCATCAGAAA	1178
35	CTCAAAAGAA GGTGATCGTT TGTCCCGCCT ACCCGTGACC TGGAAGCCCC CGCCCCGCTC	1238
	GAGTGACCCC TGACTTTCTG GACGGAACCA ACGTACTTCT TACATATATT GATTCATGTG	1298
	TCATATCTCC CTAAATGCG TAAACCAGC TGTGCCCCGA CCACCTTGGG CCCCTGCCAT	1358
40	CAGGACCTCC TGAGGCTTTG GCAAATAAAC CTCCTAAAAG GATAGAACT GAACTTGTG	1418
	GGCGGGCGCG GTGGCTCAAG CCTGTAATCC CAGCACTTTG GGAGGCCGAG GTGGGTGGAT	1478
	CACGAGGTCA GGAGATCGAG ACCATCCTGG CTAACCCGTG AAACCCCGTC TCTACTAAAA	1538
45	AAATACAAAA ATTAGCCGGG AGCGGTGGCG GCGCCTGTA GTCCAGCTA CTCGGGAGGC	1598
	TGAGGCAGGA GAATGGCGTG AACCCGGGAG GCGGAGCTTG CAGTGAGCTG AGATCCGGCC	1658
	ACTGCACTCC AGCCTGGGGG ACAGAGCGAG ACTCCGTCTC AAAAAAAAAA AAAAAAAAAA	1718
50	AAA	1721

55 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

EP 0 808 367 B1

(A) LENGTH: 345 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

10

Met Asp Arg Gly Leu Ala Leu Leu Leu Ala Gly Leu Leu Gly Leu Leu
1 5 10 15

Gln Pro Gly Cys Gly Gln Ser Leu Gln Val Lys Pro Leu Gln Val Glu
20 25 30

15

Pro Pro Glu Pro Val Val Ala Val Ala Leu Gly Ala Ser Arg Gln Leu
35 40 45

Thr Cys Arg Leu Asp Cys Ala Asp Arg Gly Ala Thr Val Gln Trp Arg
50 55 60

20

Gly Leu Asp Thr Ser Leu Gly Ala Val Gln Ser Asp Ala Gly Arg Ser
65 70 75 80

Val Leu Thr Val Arg Asn Ala Ser Leu Ser Ala Ala Gly Thr Arg Val
85 90 95

25

Cys Val Gly Ser Cys Gly Gly Arg Thr Phe Gln His Thr Val Arg Leu
100 105 110

Leu Val Tyr Ala Phe Pro Asp Gln Leu Thr Ile Ser Pro Ala Ala Leu
115 120 125

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Val Pro Gly Asp Pro Glu Val Ala Cys Thr Ala His Lys Val Thr Pro
130 135 140

Val Asp Pro Asn Ala Leu Ser Phe Ser Leu Leu Leu Gly Asp Gln Glu
145 150 155 160

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Leu Glu Gly Ala Gln Ala Leu Gly Pro Glu Val Glu Glu Glu Glu Glu
165 170 175

Glu Pro Gln Glu Glu Asp Val Leu Phe Arg Val Thr Glu Arg Trp
180 185 190

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Arg Leu Pro Thr Leu Ala Thr Pro Val Leu Pro Ala Leu Tyr Cys Gln
195 200 205

Ala Thr Met Arg Leu Pro Gly Leu Glu Leu Ser His Arg Gln Ala Ile
210 215 220

45

Pro Val Leu His Gly Pro Thr Ser Arg Glu Pro Pro Asp Thr Thr Ser
225 230 235 240

Pro Glu Pro Arg Ala Ala Thr Ser Pro Glu Thr Thr Pro Gln Gln Gly
245 250 255

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Ser Thr His Ser Pro Arg Ser Pro Gly Ser Thr Arg Thr Cys Arg Pro
 260 265 270
 5 Glu Ile Ser Gln Ala Gly Pro Thr Gln Gly Glu Val Ile Pro Thr Gly
 275 280 285
 Ser Ser Lys Pro Thr Gly Asp Gln Leu Pro Ala Ala Leu Trp Thr Ser
 290 295 300
 10 Ser Ala Val Leu Gly Leu Leu Leu Leu Ala Leu Pro Thr Tyr His Leu
 305 310 315 320
 Trp Lys Arg Cys Arg His Leu Ala Glu Asp Gly Ala His Pro Pro Ala
 325 330 335
 15 Ser Leu Ser Ser Gln Pro Phe Pro Leu
 340 345

(2) INFORMATION FOR SEQ ID NO:7:

20

(i) SEQUENCE CHARACTERISTICS:

25

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
CTCTACTGCC AGGCCACG 18

30

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

35

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
AGCCTGGGAG ATCTCAGG 19

40

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

45

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
GCCACGATGA GGCTGCCTGG 20

55

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
GTGGAGCCTG GGCTCCTGGG 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
GGAAGCTTCC ACCATGGATT TCGGACTGGC CC 32

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
CCGACTAGTG TCGGGCTGTG CAGGAC 26

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
GGACTAGTGG TTTGGACGAG CCTGTTG 27

Claims

1. An isolated nucleic acid which encodes

- (a) a naturally occurring primate MAdCAM selected from the group consisting of the protein shown in Figure 1 (SEQ ID NO: 2), the protein shown in Figure 2 (SEQ ID NO: 4), the protein shown in Figure 3 (SEQ ID NO: 6), and the mature form of any of the foregoing; or
- (b) a functional variant thereof which binds $\alpha 4\beta 7$ integrin; or
- (c) a functional fragment of a naturally occurring primate MAdCAM which binds $\alpha 4\beta 7$ integrin and comprises at least one immunoglobulin-like domain,

wherein the amino acid sequence of said functional variant is at least 75% similar to the sequence of a protein shown in Figure 1 (SEQ ID NO: 2), Figure 2 (SEQ ID NO: 4) or Figure 3 (SEQ ID NO: 6), and amino acid sequence

similarity is determined by the Clustal method with the PAM250 residue weight table, using a gap penalty of 10, a gap length penalty of 10 and pairwise alignment parameters of ktuple = 1, gap penalty = 3, window = 4, and diagonals saved = 5.

2. The isolated nucleic acid of Claim 1, wherein the isolated nucleic acid is recombinant and/or essentially pure.
3. An isolated nucleic acid of Claim 1 or Claim 2, wherein
 - (a) said nucleic acid hybridizes under high stringency conditions with a second nucleic acid, the second nucleic acid having a nucleotide sequence as shown in Figure 1 (SEQ ID NO: 1), Figure 2 (SEQ ID NO: 3) or Figure 3 (SEQ ID NO: 5) or a sequence which is the complement of the open reading frame of any one of the foregoing; or
 - (b) said nucleic acid encodes the polypeptide shown in Figure 1 (SEQ ID NO: 2), the polypeptide shown in Figure 2 (SEQ ID NO: 4), the polypeptide shown in Figure 3 (SEQ ID NO: 6), or the corresponding mature proteins.
4. The isolated nucleic acid of Claim 3 wherein said nucleic acid has a nucleotide sequence selected from the group consisting of a nucleotide sequence as shown in Figure 1 (SEQ ID NO: 1), a nucleotide sequence as shown in Figure 2 (SEQ ID NO: 3), a nucleotide sequence as shown in Figure 3 (SEQ ID NO: 5), and a portion of any of the foregoing comprising the coding sequence.
5. An isolated recombinant nucleic acid construct comprising a nucleic acid of Claim 1.
6. The isolated recombinant nucleic acid construct of Claim 5,
 - (a) wherein the recombinant nucleic acid is operably linked to an expression control sequence; or
 - (b) comprising a nucleic acid, wherein said nucleic acid encodes a polypeptide having an amino acid sequence as set forth in Figure 1 (SEQ ID NO: 2), Figure 2 (SEQ ID NO: 4), or Figure 3 (SEQ ID NO: 6); or
 - (c) said nucleic acid hybridizes under high stringency conditions with a second nucleic acid, the second nucleic acid having a nucleotide sequence as shown in Figure 1 (SEQ ID NO: 1), Figure 2 (SEQ ID NO: 3), Figure 3 (SEQ ID NO: 5) or a sequence which is the complement of the open reading frame of any one of the foregoing.
7. An isolated protein which has the amino acid sequence of:
 - (a) a naturally occurring primate MAdCAM selected from the group consisting of the protein shown in Figure 1 (SEQ ID NO: 2), the protein shown in Figure 2 (SEQ ID NO: 4), the protein shown in Figure 3 (SEQ ID NO: 6), and the mature form of any of the foregoing, or
 - (b) a functional variant thereof which binds $\alpha 4\beta 7$ integrin; or
 - (c) a functional fragment of a naturally occurring primate MAdCAM which binds $\alpha 4\beta 7$ integrin and comprises at least one immunoglobulin-like domain,

wherein the amino acid sequence of said functional variant is at least 75% similar to the sequence of a protein shown in Figure 1 (SEQ ID NO: 2), Figure 2 (SEQ ID NO: 4) or Figure 3 (SEQ ID NO: 6), and amino acid sequence similarity is determined by the Clustal method with the PAM250 residue weight table, using a gap penalty of 10, a gap length penalty of 10 and pairwise alignment parameters of ktuple = 1, gap penalty = 3, window = 4, and diagonals saved = 5.
8. The isolated protein of Claim 7, wherein
 - (a) the primate MAdCAM is a human MAdCAM, encoded by a nucleic acid which hybridizes under high stringency conditions to a second nucleic acid, the second nucleic acid having a nucleotide sequence as shown in Figure 1 (SEQ ID NO: 1), Figure 2 (SEQ ID NO: 3), Figure 3 (SEQ ID NO: 5) or a sequence which is the complement of the open reading frame of any one of the foregoing; or
 - (b) the primate MAdCAM is a human MAdCAM as shown in Figure 1 (SEQ ID NO: 2), Figure 2 (SEQ ID NO: 4), or the corresponding mature protein of either of the foregoing; or
 - (c) the primate MAdCAM is a macaque MAdCAM as shown in Figure 3 (SEQ ID NO: 6) or the corresponding mature protein.
9. An isolated naturally occurring primate MAdCAM having one or more functions selected from the group consisting of binding to $\alpha 4\beta 7$ integrin and mediation of cellular adhesion.

10. The isolated primate MAdCAM of Claim 9, wherein cellular adhesion is $\alpha 4\beta 7$ integrin-dependent.

11. The isolated primate MAdCAM of Claim 9 or Claim 10, wherein said binding is selective for $\alpha 4\beta 7$.

12. A host cell comprising a recombinant nucleic acid which encodes:

- (a) a naturally occurring primate MAdCAM selected from the group consisting of the protein shown in Figure 1 (SEQ ID NO: 2), the protein shown in Figure 2 (SEQ ID NO: 4), the protein shown in Figure 3 (SEQ ID NO: 6), and the mature form of any of the foregoing; or
- (b) a functional variant thereof which binds $\alpha 4\beta 7$ integrin; or
- (c) a functional fragment of a naturally occurring primate MAdCAM which binds $\alpha 4\beta 7$ integrin and comprises at least one immunoglobulin-like domain,

wherein the amino acid sequence of said functional variant is at least 75% similar to the sequence of a protein shown in Figure 1 (SEQ ID NO: 2), Figure 2 (SEQ ID NO: 4) or Figure 3 (SEQ ID NO: 6), and amino acid sequence similarity is determined by the Clustal method with the PAM250 residue weight table, using a gap penalty of 10, a gap length penalty of 10 and pairwise alignment parameters of ktuple = 1, gap penalty = 3, window = 4, and diagonals saved = 5.

13. The host cell of Claim 12 wherein said recombinant nucleic acid is operably linked to an expression control sequence, whereby said naturally occurring primate MAdCAM or said functional variant which binds $\alpha 4\beta 7$ integrin or said functional fragment of a naturally occurring primate MAdCAM which binds $\alpha 4\beta 7$ integrin is expressed when the host cell is maintained under conditions suitable for expression.

14. A fusion protein comprising:

- (a) a naturally occurring primate MAdCAM selected from the group consisting of the protein shown in Figure 1 (SEQ ID NO: 2), the protein shown in Figure 2 (SEQ ID NO: 4), the protein shown in Figure 3 (SEQ ID NO: 6), and the mature form of any of the foregoing, or
- (b) a functional variant thereof which binds $\alpha 4\beta 7$ integrin; or
- (c) a functional fragment of a naturally occurring primate MAdCAM which binds $\alpha 4\beta 7$ integrin and comprises at least one immunoglobulin-like domain,

wherein the amino acid sequence of said functional variant is at least 75% similar to the sequence of a protein shown in Figure 1 (SEQ ID NO: 2), Figure 2 (SEQ ID NO: 4) or Figure 3 (SEQ ID NO: 6), and amino acid sequence similarity is determined by the Clustal method with the PAM250 residue weight table, using a gap penalty of 10, a gap length penalty of 10 and pairwise alignment parameters of ktuple = 1, gap penalty = 3, window = 4, and diagonals saved = 5.

15. The fusion protein of Claim 14, comprising a first moiety and a second moiety, wherein said first moiety has the amino acid sequence of a naturally occurring primate MAdCAM or said functional variant thereof or said functional fragment and said second moiety is at least a portion of an immunoglobulin chain or variant thereof.

16. The fusion protein of Claim 15, wherein

- (a) said first moiety is joined at its C-terminal end to the N-terminal end of the second moiety; or
- (b) the first moiety is selected from the group consisting of a functional fragment of human MAdCAM containing the entire extracellular domain and a functional fragment of human MAdCAM containing two N-terminal immunoglobulin domains; or
- (c) the second moiety is at least a portion of an immunoglobulin heavy chain constant region or functional variant thereof.

17. The fusion protein of Claim 16, wherein the immunoglobulin heavy chain is of the IgG class; and/or the second moiety comprises hinge, CH2 and CH3 domains of an immunoglobulin heavy chain.

18. A hybrid immunoglobulin comprising a fusion protein as claimed in any one of Claims 14 to 17.

19. A hybrid immunoglobulin of Claim 18, wherein said hybrid immunoglobulin is a homodimer.

20. A recombinant nucleic acid construct comprising a nucleic acid containing a coding sequence which encodes a fusion protein as claimed in any one of Claims 14 to 17, wherein optionally the coding sequence is operably linked to an expression control sequence.

21. A method for producing a protein as claimed in Claim 7 comprising maintaining a host cell comprising a recombinant nucleic acid encoding said protein under conditions suitable for expression of the nucleic acid, whereby said protein is produced.

22. A method as claimed in Claim 21 further comprising

(a) introducing into a host cell a nucleic acid construct comprising a nucleic acid which encodes said protein, whereby a recombinant host cell is produced having said coding sequence operably linked to at least one expression control sequence; and

(b) maintaining the host cells produced in step (a) in a suitable medium under conditions whereby the nucleic acid is expressed.

23. The method of Claim 21 or Claim 22 further comprising the step of isolating said protein.

24. An antibody or antigen-binding fragment thereof which binds a naturally occurring primate MAdCAM, a functional variant of a naturally occurring primate MAdCAM which binds a $\alpha 4\beta 7$ integrin and/or a functional fragment of a naturally occurring primate MAdCAM which binds $\alpha 4\beta 7$ integrin and comprises at least one immunoglobulin-like domain, as defined in claim 7.

25. An antibody or antigen-binding fragment as claimed in Claim 24 for use in therapy or diagnosis.

26. Use of an antibody or antigen-binding fragment as claimed in Claim 24 for the manufacture of a medicament for treating a disease selected from the group consisting of pancreatitis, insulin-dependent diabetes mellitus, mastitis, cholecystitis, cholangitis, pericholangitis, chronic bronchitis, chronic sinusitis, asthma and graft versus host disease.

27. Use of an antibody or antigen-binding fragment as claimed in Claim 24 for the manufacture of a medicament for treating inflammatory bowel disease.

28. The antibody or antigen-binding fragment as claimed in Claim 24 or Claim 25 or the use claimed in Claim 27 wherein:

(a) said antibody or antigen-binding fragment can inhibit one or both of binding function or cellular adhesion molecule function of a naturally occurring primate MAdCAM; or

(b) said antibody or antigen-binding fragment can selectively inhibit $\alpha 4\beta 7$ -dependent adhesion, or

(c) said antibody or antigen-binding fragment can inhibit the binding of a naturally occurring primate MAdCAM to $\alpha 4\beta 7$ integrin.

29. The antibody or antigen-binding fragment as claimed in Claim 24, Claim 25 or Claim 28 or the use claimed in Claim 27 or Claim 28, wherein said antibody or antigen-binding fragment is a monoclonal antibody or antigen-binding fragment thereof.

30. The antibody or antigen-binding fragment claimed in Claim 24, 25, 28 or 29 or the use claimed in any one of Claims 27 to 29, wherein said naturally occurring primate MAdCAM is a naturally occurring human MAdCAM.

31. Use of a primate MAdCAM, a functional variant thereof which binds $\alpha 4\beta 7$ integrin, or a hybrid immunoglobulin comprising a primate MAdCAM or $\alpha 4\beta 7$ integrin-binding variant thereof, for the manufacture of a medicament for treating a disease selected from the group consisting of pancreatitis, insulin-dependent diabetes mellitus, mastitis, cholecystitis, cholangitis, pericholangitis, chronic bronchitis, chronic sinusitis, asthma and graft versus host disease, wherein said primate MAdCAM is a protein having the amino acid sequence of a naturally occurring primate MAdCAM selected from the group consisting of the protein shown in Figure 1 (SEQ ID NO: 2), the protein shown in Figure 2 (SEQ ID NO: 4), the protein shown in Figure 3 (SEQ ID NO: 6), and the mature form of any of the foregoing, or a functional portion thereof which binds $\alpha 4\beta 7$ integrin, said functional portion comprises at least one immunoglobulin-like domain, and

wherein the amino acid sequence of said functional variant is at least 75% similar to the sequence of a protein shown in Figure 1 (SEQ ID NO: 2), Figure 2 (SEQ ID NO: 4) or Figure 3 (SEQ ID NO: 6), and amino acid sequence

similarity is determined by the Clustal method with the PAM250 residue weight table, using a gap penalty of 10, a gap length penalty of 10 and pairwise alignment parameters of ktuple = 1, gap penalty = 3, window = 4, and diagonals saved = 5.

5 **32.** Use of a primate MAdCAM, a functional variant thereof which binds $\alpha 4\beta 7$ integrin, or a hybrid immunoglobulin comprising a primate MAdCAM or $\alpha 4\beta 7$ integrin-binding variant thereof, for the manufacture of a medicament for treating inflammatory bowel disease,
 wherein said primate MAdCAM is a protein having the amino acid sequence of a naturally occurring primate MAdCAM selected from the group consisting of the protein shown in Figure 1 (SEQ ID NO: 2), the protein shown in Figure 2 (SEQ ID NO: 4), the protein shown in Figure 3 (SEQ ID NO: 6), and the mature form of any of the foregoing, or a functional portion thereof which binds $\alpha 4\beta 7$ integrin, said functional portion comprises at least one immunoglobulin-like domain, and
 wherein the amino acid sequence of said functional variant is at least 75% similar to the sequence of a protein shown in Figure 1 (SEQ ID NO:2), Figure 2 (SEQ ID NO:4) or Figure 3 (SEQ ID NO:6), and amino acid sequence similarity is determined by the Clustal method with the PAM250 residue weight table, using a gap penalty of 10, a gap length penalty of 10 and pairwise alignment parameters of ktuple = 1, gap penalty = 3, window = 4, and diagonals saved = 5.

20 **33.** Use according to Claim 32 wherein

- (a) the primate MAdCAM, functional variant or hybrid immunoglobulin can inhibit the interaction of a primate MAdCAM with an $\alpha 4\beta 7$ integrin; or
- (b) said hybrid immunoglobulin contains a fusion protein, comprising a first moiety and a second moiety, wherein said first moiety is a primate MAdCAM and said second moiety is at least a portion of an immunoglobulin chain.

25 **34.** Use of an antibody or antigen-binding fragment thereof which binds the $\beta 7$ chain of $\alpha 4\beta 7$ integrin and inhibits binding of primate MAdCAM to $\alpha 4\beta 7$ integrin for the manufacture of a medicament for treating a disease selected from the group consisting of pancreatitis, insulin-dependent diabetes mellitus, mastitis, cholecystitis, cholangitis, pericholangitis, chronic bronchitis, chronic sinusitis, asthma and graft versus host disease,
 wherein said primate MAdCAM is (a) a naturally occurring primate MAdCAM selected from the group consisting of the protein shown in Figure 1 (SEQ ID NO: 2), the protein shown in Figure 2 (SEQ ID NO: 4), the protein shown in Figure 3 (SEQ ID NO: 6), and the mature form of any of the foregoing, or
 (b) a functional variant thereof which binds $\alpha 4\beta 7$ integrin, and
 wherein the amino acid sequence of said functional variant is at least 75% similar to the sequence of a protein shown in Figure 1 (SEQ ID NO: 2), Figure 2 (SEQ ID NO: 4) or Figure 3 (SEQ ID NO: 6), and amino acid sequence similarity is determined by the Clustal method with the PAM250 residue weight table, using a gap penalty of 10, a gap length penalty of 10 and pairwise alignment parameters of ktuple = 1, gap penalty = 3, window = 4, and diagonals saved = 5.

40 **35.** Use of an antibody or antigen-binding fragment thereof which binds the $\beta 7$ chain of $\alpha 4\beta 7$ integrin and inhibits binding of MAdCAM to $\alpha 4\beta 7$ integrin for the manufacture of a medicament for treating inflammatory bowel disease.

36. Use according to Claim 35 wherein the antibody or antigen-binding fragment thereof inhibits adhesion of leukocytes expressing an integrin containing the $\beta 7$ chain and endothelium expressing MAdCAM.

45 **37.** Use according to Claim 35 or 36 wherein

- (a) more than one antibody or antigen-binding fragment thereof which inhibits the binding of leukocytes to endothelial MAdCAM is employed; or
- (b) more than one antibody or antigen-binding fragment thereof which inhibits the binding of leukocytes to endothelial ligands is employed, and at least one antibody or fragment inhibits the binding of leukocytes to an endothelial ligand other than MAdCAM.

55 **38.** Use according to Claim 35 wherein the antibody or antigen-binding fragment inhibits the binding of leukocytes to endothelial MAdCAM.

39. Use according to any one of Claims 27-30 wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a chimeric antibody, a chimeric antigen-binding fragment, a humanized antibody, and

humanized antigen-binding fragment.

- 5 **40.** Use according to any one of Claims 27-30 wherein the disease is ulcerative colitis, Crohn's disease, Celiac disease, enteropathy associated with seronegative arthropathies, microscopic or collagenous colitis, eosinophilic gastroenteritis, or pouchitis.
- 10 **41.** Use of an antibody or antigen-binding fragment thereof which binds $\alpha 4\beta 7$ integrin for the manufacture of a medicament for treating a disease selected from the group consisting of pancreatitis, insulin-dependent diabetes mellitus, mastitis, cholecystitis, cholangitis, pericholangitis, chronic bronchitis, chronic sinusitis, asthma and graft versus host disease, wherein said antibody or antigen-binding fragment has the epitopic specificity of the ACT-1 monoclonal antibody.
- 15 **42.** The use of any one of Claims 26, 31, 34 and 41, wherein said disease is pancreatitis or insulin-dependent diabetes mellitus.
- 20 **43.** Use of an antibody or antigen-binding fragment thereof which binds $\alpha 4\beta 7$ integrin for the manufacture of a medicament for treating inflammatory bowel disease in a human, wherein said antibody or antigen-binding fragment has the epitopic specificity of the ACT-1 monoclonal antibody.
- 25 **44.** Use of any one of Claims 35-38 and 43 wherein said inflammatory bowel disease is selected from the group consisting of ulcerative colitis, Crohn's disease, Celiac disease, enteropathy associated with seronegative arthropathies, microscopic or collagenous colitis, eosinophilic gastroenteritis and pouchitis.
- 30 **45.** Use of any one of Claims 35-38, 43 and 44 wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a chimeric antibody, a chimeric antigen-binding fragment, a humanized antibody, and humanized antigen-binding fragment.
- 35 **46.** Use of any one of Claims 35-38, 43 and 44 wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody or antigen-binding fragment thereof.
- 40 **47.** A method of detecting a selected primate MAdCAM in a sample comprising:
 (a) contacting a sample with an antibody or antigen-binding fragment thereof of claim 24 under conditions suitable for specific binding of said antibody or fragment thereto; and
 (b) detecting the formation of a complex between said antibody or fragment and MAdCAM.
- 45 **48.** A method of detecting or identifying a ligand of or an agent which binds a primate MAdCAM comprising combining an agent to be tested with an isolated protein of Claim 7 or host cell of Claim 12 under conditions suitable for binding of ligand thereto, and detecting or measuring the formation of a complex between said agent and said protein.
- 50 **49.** A method of detecting an inhibitor of binding of a naturally occurring primate MAdCAM to a ligand thereof comprising:
 (a) combining an agent to be tested with a ligand of said primate MAdCAM and a composition comprising an isolated protein as claimed in Claim 7 or host cell as claimed in Claim 12 under conditions suitable for binding of ligand thereto; and
 (b) detecting or measuring binding between said protein or host cell and ligand, whereby decreased binding as compared with a suitable control indicates that the agent is an inhibitor.
- 55 **50.** A method of detecting an inhibitor of binding of a naturally occurring primate MAdCAM to a ligand thereof comprising:
 (a) combining an agent to be tested with a ligand of said primate MAdCAM and a hybrid immunoglobulin of Claim 18 or 19 or a fusion protein of any one of Claims 14 to 17, under conditions suitable for binding of ligand to a naturally occurring primate MAdCAM; and
 (b) detecting or measuring binding between said hybrid immunoglobulin or fusion protein and said ligand, whereby decreased binding as compared with a suitable control indicates that the agent is an inhibitor.
- 60 **51.** The method of Claim 50, wherein said ligand is $\alpha 4\beta 7$ integrin, and an agent to be tested, a cell expressing $\alpha 4\beta 7$ integrin; and a hybrid immunoglobulin of claim 18 or a fusion protein of claim 14 or claim 15 are combined.

52. A method of detecting an inhibitor of cellular adhesion mediated by MAdCAM, comprising:

- (a) combining an agent to be tested, a host cell of Claim 12 and a second cell bearing an $\alpha 4\beta 7$ integrin under conditions suitable for adhesion of said host cell to said second cell; and
 (b) detecting or measuring adhesion between said host and second cells, whereby decreased adhesion as compared with a suitable control indicates that the agent is an inhibitor.

53. The method of any one of Claims 48 to 52, wherein the agent is an antibody or antigen-binding fragment thereof.

54. The isolated nucleic acid of Claim 4, wherein the nucleic acid is operably linked to an expression control sequence.

55. The use of any one of Claims 26, 31, 34 and 41, wherein said disease is mastitis.

56. The use of any one of Claims 26, 31, 34 and 41, wherein said disease is cholecystitis, cholangitis or pericholangitis.

57. The use of any one of Claims 26, 31, 34 and 41, wherein said disease is chronic bronchitis, chronic sinusitis or asthma.

58. The use of any one of Claims 26, 31, 34 and 41, wherein said disease is graft versus host disease.

59. Use according to any one of claims 26, 34 and 41, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a chimeric antibody, a chimeric antigen-binding fragment, a humanized antibody, and humanized antigen-binding fragment.

Patentansprüche

1. Eine isolierte Nucleinsäure, welche codiert

- (a) ein natürlich vorkommendes MAdCAM von Primaten, ausgewählt aus der Gruppe in Figur 1 (SEQ ID NO: 2) gezeigtes Protein, in Figur 2 (SEQ ID NO: 4) gezeigtes Protein, in Figur 3 (SEQ ID NO: 6) gezeigtes Protein und reifes Protein von jedem der vorstehenden Proteine; oder
 (b) eine funktionelle Variante derselben, welche $\alpha 4\beta 7$ -Integrin bindet; oder
 (c) ein funktionelles Fragment eines natürlich vorkommenden MAdCAM von Primaten, welches $\alpha 4\beta 7$ -Integrin bindet und mindestens eine Immunglobulin-ähnliche Domäne umfasst,

wobei die Aminosäuresequenz der funktionellen Variante mit der Sequenz eines in Figur 1 (SEQ ID NO: 2), in Figur 2 (SEQ ID NO: 4) oder in Figur 3 (SEQ ID NO: 6) gezeigten Proteins eine 75% Ähnlichkeit aufweist und die Ähnlichkeit in der Aminosäuresequenz mittels der "Clustal method with the PAM250 residue weight table" unter Verwendung einer Gap Penalty von 10, einer Gap Length Penalty von 10 und den "pairwise alignment parameters" k-tuple = 1, Gap Penalty = 3, Window = 4 und Diagonals saved = 5 ermittelt wird.

2. Isolierte Nucleinsäure nach Anspruch 1, wobei die isolierte Nucleinsäure rekombinant und/oder im Wesentlichen rein ist.

3. Isolierte Nucleinsäure nach Anspruch 1 oder 2, wobei

- (a) die Nucleinsäure unter hoch stringenten Bedingungen mit einer zweiten Nucleinsäure hybridisiert, wobei die zweite Nucleinsäure eine Nucleotidsequenz wie in Figur 1 (SEQ ID NO: 1), in Figur 2 (SEQ ID NO: 3) oder in Figur 3 (SEQ ID NO: 5) gezeigt aufweist oder eine Sequenz hat, welche komplementär zu dem offenen Leseraster von jeder der obigen Nucleinsäuren ist; oder
 (b) die Nucleinsäure das in Figur 1 (SEQ ID NO: 2) gezeigte Polypeptid, das in Figur 2 (SEQ ID NO: 4) gezeigte Polypeptid, das in Figur 3 (SEQ ID NO: 6) gezeigte Polypeptid oder die entsprechenden reifen Proteine codiert.

4. Isolierte Nucleinsäure nach Anspruch 3, wobei die Nucleinsäure eine Nucleotidsequenz aufweist, die ausgewählt ist aus der Gruppe Nucleotidsequenz wie in Figur 1 (SEQ ID NO: 1), Nucleotidsequenz wie in Figur 2 (SEQ ID NO: 3) oder Nucleotidsequenz wie in Figur 3 (SEQ ID NO: 5) gezeigt und ein Abschnitt von jeder der obigen, die codierende Sequenz umfassenden Nucleotidsequenzen.

5. Isoliertes rekombinantes Nucleinsäure-Konstrukt, das eine Nucleinsäure des Anspruchs 1 umfasst.

6. Isoliertes rekombinantes Nucleinsäure-Konstrukt nach Anspruch 5, wobei

- (a) die rekombinante Nucleinsäure funktionsfähig an eine Kontrollsequenz für die Expression gebunden ist; oder
- (b) das Nucleinsäure-Konstrukt eine Nucleinsäure umfasst, wobei diese Nucleinsäure ein Polypeptid mit einer Aminosäuresequenz wie in Figur 1 (SEQ ID NO: 2), wie in Figur 2 (SEQ ID NO: 4) oder wie in Figur 3 (SEQ ID NO: 6) codiert; oder
- (c) diese Nucleinsäure unter hoch stringenten Bedingungen mit einer zweiten Nucleinsäure hybridisiert wobei die zweite Nucleinsäure eine Nucleotidsequenz wie in Figur 1 (SEQ ID NO: 1), in Figur 2 (SEQ ID NO: 3), in Figur 3 (SEQ ID NO: 5) gezeigt aufweist oder eine Sequenz hat, welche komplementär zu dem offenen Leseraster von jeder der obigen Nucleinsäuren ist.

7. Ein isoliertes Protein mit der Aminosäuresequenz

- (a) eines natürlich vorkommenden MAdCAM von Primaten, ausgewählt aus der Gruppe in Figur 1 (SEQ ID NO: 2) gezeigtes Protein, in Figur 2 (SEQ ID NO: 4) gezeigtes Protein, in Figur 3 (SEQ ID NO: 6) gezeigtes Protein und die reife Form von jedem der vorstehenden Proteine; oder
- (b) einer funktionellen Variante derselben, welche $\alpha 4\beta 7$ -Integrin bindet; oder
- (c) eines funktionellen Fragments eines natürlich vorkommenden MAdCAM von Primaten, welches $\alpha 4\beta 7$ -Integrin bindet und mindestens eine Immunglobulin-ähnliche Domäne umfasst,

wobei die Aminosäuresequenz der funktionellen Variante mit der Sequenz eines in Figur 1 (SEQ ID NO: 2), in Figur 2 (SEQ ID NO: 4) oder in Figur 3 (SEQ ID NO: 6) gezeigten Proteins eine 75% Ähnlichkeit aufweist und die Ähnlichkeit in der Aminosäuresequenz mittels der "Clustal method with the PAM250 residue weight table" unter Verwendung einer Gap Penalty von 10, einer Gap Length Penalty von 10 und "pairwise alignment parameters" mit k-tuple = 1, Gap Penalty = 3, Window = 4 und Diagonals saved = 5 ermittelt wird.

8. Isoliertes Protein nach Anspruch 7, wobei

- (a) das MAdCAM von Primaten ein humanes MAdCAM ist, das von einer Nucleinsäure codiert wird, welche unter hoch stringenten Bedingungen an eine zweite Nucleinsäure hybridisiert, wobei die zweite Nucleinsäure eine Nucleotidsequenz wie in Figur 1 (SEQ ID NO: 1), in Figur 2 (SEQ ID NO: 3) oder in Figur 3 (SEQ ID NO: 5) gezeigt aufweist oder eine Sequenz hat, welche komplementär zu dem offenen Leseraster von jeder der obigen Nucleinsäuren ist; oder
- (b) das MAdCAM von Primaten ein humanes MAdCAM wie das in Figur 1 (SEQ ID NO: 2) gezeigte, das in Figur 2 (SEQ ID NO: 4) gezeigte oder das entsprechende reife Proteine von jedem der vorstehenden ist; oder
- (c) das MAdCAM von Primaten ein Makaken-MAdCAM wie in Figur 3 (SEQ ID NO: 6) oder das entsprechende reife Protein ist.

9. Isoliertes natürlich vorkommendes MAdCAM von Primaten mit einer oder mehreren Funktionen, die ausgewählt sind aus der Gruppe Bindung an $\alpha 4\beta 7$ -Integrin und Vermittlung einer Zelladhäsion.

10. Isoliertes MAdCAM von Primaten nach Anspruch 9, wobei die Zelladhäsion abhängig von $\alpha 4\beta 7$ -Integrin ist.

11. Isoliertes MAdCAM von Primaten nach Anspruch 9 oder 10, wobei die Bindung selektiv für $\alpha 4\beta 7$ ist.

12. Wirtszelle mit einer rekombinanten Nucleinsäure, welche

- (a) ein natürlich vorkommendes MAdCAM von Primaten codiert, ausgewählt aus der Gruppe in Figur 1 (SEQ ID NO: 2) gezeigtes Protein, in Figur 2 (SEQ ID NO: 4) gezeigtes Protein, in Figur 3 (SEQ ID NO: 6) gezeigtes Protein und reifes Protein von jedem der vorstehenden Proteine; oder
- (b) eine funktionelle Variante derselben codiert, welche $\alpha 4\beta 7$ -Integrin bindet; oder
- (c) ein funktionelles Fragment eines natürlich vorkommenden MAdCAM von Primaten codiert, welches $\alpha 4\beta 7$ -Integrin bindet und mindestens eine Immunglobulin-ähnliche Domäne umfasst,

wobei die Aminosäuresequenz der funktionellen Variante mit der Sequenz eines in Figur 1 (SEQ ID NO: 2), in Figur 2 (SEQ ID NO: 4) oder in Figur 3 (SEQ ID NO: 6) gezeigten Proteins eine 75% Ähnlichkeit aufweist und die Ähnlichkeit

in der Aminosäuresequenz mittels der "Clustal method with the PAM250 residue weight table" unter Verwendung einer Gap Penalty von 10, einer Gap Length Penalty von 10 und den "pairwise alignment parameters" k-tuple = 1, Gap Penalty = 3, Window = 4 und Diagonals saved = 5 ermittelt wird.

13. Wirtszelle nach Anspruch 12, in welcher die rekombinante Nucleinsäure funktionsfähig an eine Kontrollsequenz für die Expression gebunden ist, wobei das natürlich vorkommende MAdCAM von Primaten oder die funktionelle Variante, die $\alpha 4\beta 7$ -Integrin bindet oder das funktionelle Fragment eines natürlich vorkommenden MAdCAM von Primaten, das $\alpha 4\beta 7$ -Integrin bindet, exprimiert wird, wenn die Wirtszelle unter für die Expression geeigneten Bedingungen gehalten wird.

14. Fusionsprotein mit

- (a) einem natürlich vorkommenden MAdCAM von Primaten, ausgewählt aus der Gruppe in Figur 1 (SEQ ID NO: 2) gezeigtes Protein, in Figur 2 (SEQ ID NO: 4) gezeigtes Protein, in Figur 3 (SEQ ID NO: 6) gezeigtes Protein und das reife Protein von jedem der vorstehenden Proteine; oder
- (b) einer funktionellen Variante derselben, welche $\alpha 4\beta 7$ -Integrin bindet; oder
- (c) einem funktionellen Fragment eines natürlich vorkommenden MAdCAM von Primaten, welches $\alpha 4\beta 7$ -Integrin bindet und mindestens eine Immunglobulin-ähnliche Domäne umfasst,

wobei die Aminosäuresequenz der funktionellen Variante mit der Sequenz eines in Figur 1 (SEQ ID NO: 2), in Figur 2 (SEQ ID NO: 4) oder in Figur 3 (SEQ ID NO: 6) gezeigten Proteins eine 75% Ähnlichkeit aufweist und die Ähnlichkeit in der Aminosäuresequenz mittels der "Clustal method with the PAM250 residue weight table" unter Verwendung einer Gap Penalty von 10, einer Gap Length Penalty von 10 und den "pairwise alignment parameters" k-tuple = 1, Gap Penalty = 3, Window = 4 und Diagonals saved = 5 ermittelt wird.

15. Fusionsprotein nach Anspruch 14 mit einem ersten Anteil und einem zweiten Anteil, wobei der erste Anteil die Aminosäuresequenz eines natürlich vorkommenden MAdCAM von Primaten aufweist oder die funktionelle Variante desselben oder das funktionelle Fragment und der zweite Anteil zumindest einen Teil einer Immunglobulinkette oder eine Variante derselben darstellt.

16. Fusionsprotein nach Anspruch 15, wobei

- (a) der erste Anteil an seinem C-terminalen Ende mit dem N-terminalen Ende des zweiten Anteils verbunden ist; oder
- (b) der erste Anteil ausgewählt ist aus der Gruppe die ganze extrazelluläre Domäne enthaltendes funktionelles Fragment des MAdCAM vom Menschen und zwei N-terminale Immunglobulin-Domänen enthaltendes funktionelles Fragment des MAdCAM vom Menschen; oder
- (c) der zweite Anteil mindestens ein Abschnitt einer konstanten Region einer schweren Immunglobulinkette oder eine funktionelle Variante derselben darstellt.

17. Fusionsprotein nach Anspruch 16, wobei die schwere Immunglobulinkette von der IgG-Klasse ist und/oder der zweite Anteil die Gelenk-, C_H2 und C_H3-Domäne der schweren Immunglobulinkette umfasst.

18. Immunglobulin-Hybrid mit einem Fusionsprotein nach jedem der Ansprüche 14 bis 17.

19. Immunglobulin-Hybrid nach Anspruch 18, wobei das Immunglobulin-Hybrid ein Homodimer ist.

20. Rekombinantes Nucleinsäure-Konstrukt, welches eine kodierende Sequenz enthält, die ein Fusionsprotein nach jedem der Ansprüche 14 bis 17 codiert, wobei gegebenenfalls die codierende Sequenz mit einer Kontrollsequenz für die Expression funktionsfähig verbunden ist.

21. Verfahren zur Gewinnung eines Proteins gemäß Anspruch 7, welches den Schritt umfasst, dass eine Wirtszelle, welche eine dieses Protein codierende rekombinante Nucleinsäure umfasst, unter für die Expression dieser Nucleinsäure geeigneten Bedingungen gehalten wird, wodurch das Protein produziert wird.

22. Verfahren nach Anspruch 21 mit den weiteren Schritten

- (a) Einschleusen eines Nucleinsäure-Konstrukts, welches eine das Protein codierende Nucleinsäure umfasst,

in eine Wirtszelle, wodurch eine rekombinante Wirtszelle gewonnen wird, in welcher die codierende Sequenz mit mindestens einer Kontrollsequenz für die Expression funktionsfähig verbunden ist und
(b) Halten der in Schritt (a) erzeugten Wirtszelle in einem geeigneten Medium unter Bedingungen, bei welchen die Nucleinsäure exprimiert wird.

23. Verfahren nach Anspruch 21 oder 22 mit dem Schritt der Isolierung des Proteins.

24. Antikörper oder Antigen-bindendes Fragment desselben, welches ein natürlich vorkommendes MAdCAM von Primaten, eine funktionelle Variante eines natürlich vorkommenden MAdCAM von Primaten, welches $\alpha 4\beta 7$ -Integrin bindet, und/oder ein funktionelles Fragment eines natürlich vorkommenden MAdCAM von Primaten, welches $\alpha 4\beta 7$ -Integrin bindet und mindestens, wie in Anspruch 7 angegeben, eine Immunglobulin-ähnliche Domäne umfasst, bindet.

25. Antikörper oder Antigen-bindendes Fragment nach Anspruch 24 zur Verwendung in der Therapie oder Diagnose.

26. Verwendung eines Antikörpers oder eines Antigen-bindenden Fragments nach Anspruch 24 für die Herstellung eines Medikaments zur Behandlung einer Krankheit, die ausgewählt ist aus der Gruppe Pankreatitis, Insulin-abhängiger Diabetes mellitus, Mastitis, Cholezystitis, Cholangitis, Pericholangitis, chronische Bronchitis, chronische Sinusitis, Asthma und Transplantat-Wirt-Reaktion.

27. Verwendung eines Antikörpers oder eines Antigen-bindenden Fragments nach Anspruch 24 für die Herstellung eines Medikaments zur Behandlung einer entzündlichen Darmerkrankung.

28. Antikörpers oder Antigen-bindendes Fragment nach Anspruch 24 oder 25 oder Verwendung nach Anspruch 27, wobei

(a) der Antikörper oder das Antigen-bindende Fragment die Bindungsfunktion oder die zelluläre Adhäsionsmolekül-Funktion oder beide Funktionen eines natürlich vorkommenden MAdCAM von Primaten hemmen können; oder

(b) der Antikörper oder das Antigen-bindende Fragment die $\alpha 4\beta 7$ -abhängige Adhäsion selektiv hemmen können; oder

(c) der Antikörper oder das Antigen-bindende Fragment die Bindung eines natürlich vorkommenden MAdCAM von Primaten an das $\alpha 4\beta 7$ -Integrin hemmen können.

29. Antikörper oder Antigen-bindendes Fragment nach den Ansprüchen 24, 25 oder 28 oder Verwendung nach Anspruch 27 oder 28, wobei der Antikörper oder das Antigen-bindende Fragment ein monoklonaler Antikörper oder ein monoklonales Antigen-bindendes Fragment sind.

30. Antikörper oder Antigen-bindendes Fragment nach den Ansprüchen 24, 25, 28 oder 29 oder Verwendung nach jedem der Ansprüche 27 bis 29, wobei das natürlich vorkommende MAdCAM von Primaten ein natürlich vorkommendes MAdCAM des Menschen ist.

31. Verwendung eines MAdCAM von Primaten, einer $\alpha 4\beta 7$ -Integrin bindenden funktionellen Variante desselben oder eines Immunglobulin-Hybrids mit einem MAdCAM von Primaten oder einer $\alpha 4\beta 7$ -Integrin-bindenden Variante desselben für die Herstellung eines Medikaments zur Behandlung einer Krankheit, die ausgewählt ist aus der Gruppe Pankreatitis, Insulin-abhängiger Diabetes mellitus, Mastitis, Cholezystitis, Cholangitis, Pericholangitis, chronische Bronchitis, chronische Sinusitis, Asthma und Transplantat-Wirt-Reaktion; wobei das MAdCAM von Primaten ein Protein mit der Aminosäuresequenz eines natürlich vorkommenden MAdCAM von Primaten ist, das ausgewählt ist aus der Gruppe in Figur 1 (SEQ ID NO: 2) gezeigtes Protein, in Figur 2 (SEQ ID NO: 4) gezeigtes Protein, in Figur 3 (SEQ ID NO: 6) gezeigtes Protein und die reife Form von jedem der vorstehenden Proteine oder ein funktioneller Abschnitt derselben, welcher $\alpha 4\beta 7$ -Integrin-bindet, wobei der funktionelle Abschnitt mindestens eine Immunglobulin-ähnliche Domäne umfasst, und wobei die Aminosäuresequenz der funktionellen Variante mit der Sequenz eines in Figur 1 (SEQ ID NO: 2), in Figur 2 (SEQ ID NO: 4) oder in Figur 3 (SEQ ID NO: 6) gezeigten Proteins eine 75% Ähnlichkeit aufweist und die Ähnlichkeit in der Aminosäuresequenz mittels der "Clustal method with the PAM250 residue weight table" unter Verwendung einer Gap Penalty von 10, einer Gap Length Penalty von 10 und den "pairwise alignment parameters" k-tuple = 1, Gap Penalty = 3, Window = 4 und Diagonals saved = 5 ermittelt wird.

32. Verwendung eines MAdCAM von Primaten, einer $\alpha 4\beta 7$ -Integrin bindenden funktionellen Variante desselben oder eines Immunglobulin-Hybrids mit einem MAdCAM von Primaten oder einer $\alpha 4\beta 7$ -Integrin-bindenden Variante desselben für die Herstellung eines Medikaments zur Behandlung einer entzündlichen Darmerkrankung, wobei das MAdCAM von Primaten ein Protein mit der Aminosäuresequenz eines natürlich vorkommenden MAdCAM von Primaten ist, das ausgewählt ist aus der Gruppe in Figur 1 (SEQ ID NO: 2) gezeigtes Protein, in Figur 2 (SEQ ID NO: 4) gezeigtes Protein, in Figur 3 (SEQ ID NO: 6) gezeigtes Protein und die reife Form von jedem der vorstehenden Proteine oder ein funktioneller Abschnitt derselben, welcher $\alpha 4\beta 7$ -Integrin-bindet, wobei der funktionelle Abschnitt mindestens eine Immunglobulin-ähnliche Domäne umfasst, und wobei die Aminosäuresequenz der funktionellen Variante mit der Sequenz eines in Figur 1 (SEQ ID NO: 2), in Figur 2 (SEQ ID NO: 4) oder in Figur 3 (SEQ ID NO: 6) gezeigten Proteins eine 75% Ähnlichkeit aufweist und die Ähnlichkeit in der Aminosäuresequenz mittels der "Clustal method with the PAM250 residue weight table" unter Verwendung einer Gap Penalty von 10, einer Gap Length Penalty von 10 und den "pairwise alignment parameters" k-tuple = 1, Gap Penalty = 3, Window = 4 und Diagonals saved = 5 ermittelt wird.

33. Verwendung nach Anspruch 32 wobei

(a) das MAdCAM von Primaten, die funktionelle Variante oder das Immunglobulin-Hybrid die Wechselwirkung von einem MAdCAM von Primaten mit einem $\alpha 4\beta 7$ -Integrin hemmen können; oder

(b) das Immunglobulin-Hybrid ein Fusionsprotein enthält, das einen ersten und einen zweiten Anteil umfasst, wobei der erste Anteil ein MAdCAM von Primaten ist und der zweite Anteil mindestens ein Abschnitt einer Immunglobulinkette.

34. Verwendung eines Antikörpers oder eines Antigen-bindenden Fragments desselben, welches die $\beta 7$ -Kette des $\alpha 4\beta 7$ -Integrins bindet und die Bindung des MAdCAM von Primaten an $\alpha 4\beta 7$ -Integrin hemmt, für die Herstellung eines Medikaments zur Behandlung einer Krankheit, die ausgewählt ist aus der Gruppe Pankreatitis, Insulin-abhängiger Diabetes mellitus, Mastitis, Cholezystitis, Cholangitis, Pericholangitis, chronische Bronchitis, chronische Sinusitis, Asthma und Transplantat-Wirt-Reaktion;

wobei das MAdCAM von Primaten (a) ein natürlich vorkommendes MAdCAM von Primaten ist, ausgesucht aus der Gruppe in Figur 1 (SEQ ID NO: 2) gezeigtes Protein, in Figur 2 (SEQ ID NO: 4) gezeigtes Protein, in Figur 3 (SEQ ID NO: 6) gezeigtes Protein und die reife Form von jedem der vorstehenden Proteine oder (b) eine $\alpha 4\beta 7$ -Integrin bindende funktionelle Variante desselben ist; und

wobei die Aminosäuresequenz der funktionellen Variante mit der Sequenz eines in Figur 1 (SEQ ID NO: 2), in Figur 2 (SEQ ID NO: 4) oder in Figur 3 (SEQ ID NO: 6) gezeigten Proteins eine 75% Ähnlichkeit aufweist und die Ähnlichkeit in der Aminosäuresequenz mittels der "Clustal method with the PAM250 residue weight table" unter Verwendung einer Gap Penalty von 10, einer Gap Length Penalty von 10 und den "pairwise alignment parameters" k-tuple = 1, Gap Penalty = 3, Window = 4 und Diagonals saved = 5 ermittelt wird.

35. Verwendung eines Antikörpers oder eines Antigen-bindenden Fragments desselben, welches die $\beta 7$ -Kette des $\alpha 4\beta 7$ -Integrins bindet und die Bindung des MAdCAM von Primaten an $\alpha 4\beta 7$ -Integrin hemmt, für die Herstellung eines Medikaments zur Behandlung einer entzündlichen Darmkrankheit.

36. Verwendung nach Anspruch 35, wobei der Antikörper oder das Antigen-bindende Fragment desselben die Adhäsion von Leukozyten, die ein die $\beta 7$ -Kette enthaltendes Integrin exprimieren sowie MAdCAM exprimierendes Endothel hemmen.

37. Verwendung nach Anspruch 35 oder 36, wobei

(a) mehr als ein Antikörper oder Antigen-bindendes Fragment desselben, welche die Bindung von Leukozyten an Endothel-MAdCAM hemmen, verwendet wird; oder

(b) mehr als ein Antikörper oder Antigen-bindendes Fragment desselben, welche die Bindung von Leukozyten an Endothel-Liganden hemmen, verwendet wird und mindestens ein Antikörper oder Fragment die Bindung von Leukozyten an einen anderen Endothel-Liganden als MAdCAM hemmt.

38. Verwendung nach Anspruch 35, wobei der Antikörper oder das Antigen-bindende Fragment die Bindung von Leukozyten an Endothel-MAdCAM hemmen.

39. Verwendung nach jedem der Ansprüche 27 bis 30, wobei der Antikörper oder das Antigen-bindende Fragment desselben ausgewählt sind aus der Gruppe chimärer Antikörper, chimäres Antigen-bindendes Fragment, humani-

sierter Antikörper und humanisiertes Antigen-bindendes Fragment.

40. Verwendung nach jedem der Ansprüche 27 bis 30, wobei die Krankheit Colitis ulcerosa, Morbus Crohn, Zöliakie, eine mit einer seronegativen Gelenkerkrankung einhergehende Darmerkrankung, eine mikroskopische oder Colla-
gen produzierende Colitis, eine eosinophile Gastroenteritis oder Pouchitis ist.

41. Verwendung eines Antikörpers oder eines Antigen-bindenden Fragments desselben, welches $\alpha 4 \beta 7$ -Integrin bindet, für die Herstellung eines Medikaments zur Behandlung einer Krankheit, die ausgewählt ist aus der Gruppe Pan-
kreatitis, Insulin-abhängiger Diabetes mellitus, Mastitis, Cholezystitis, Cholangitis, Pericholangitis, chronische Bron-
chitis, chronische Sinusitis, Asthma und Transplantat-Wirt-Reaktion, wobei der Antikörper oder das Antigen-bin-
dende Fragment über die Epitopspezifität des monoklonalen Antikörpers Act-1 verfügen.

42. Verwendung nach jedem der Ansprüche 26, 31, 34 und 41, wobei die Krankheit Pancreatitis oder Insulin abhängiger
Diabetes mellitus ist.

43. Verwendung eines Antikörpers oder eines Antigen-bindenden Fragments desselben, welches $\alpha 4 \beta 7$ -Integrin bindet, für die Herstellung eines Medikaments zur Behandlung einer entzündlichen Darmerkrankung beim Menschen, wobei
der Antikörper oder das Antigen-bindende Fragment über die Epitopspezifität des monoklonalen Antikörpers Act-1
verfügen.

44. Verwendung nach jedem der Ansprüche 35 bis 38 und 43, wobei die entzündliche Darmerkrankung ausgewählt ist
aus der Gruppe Colitis ulcerosa, Morbus Crohn, Zöliakie, mit einer seronegativen Gelenkerkrankung einhergehende
Darmerkrankung, mikroskopische oder Collagen produzierende Colitis, eine eosinophile Gastroenteritis oder Pou-
chitis

45. Verwendung nach jedem der Ansprüche 35 bis 38, 43 und 44, wobei der Antikörper oder das Antigen-bindende
Fragment desselben ausgewählt sind aus der Gruppe chimärer Antikörper, chimäres Antigen-bindendes Fragment,
humanisierter Antikörper und humanisiertes Antigen-bindendes Fragment.

46. Verwendung nach jedem der Ansprüche 35 bis 38, 43 und 44, wobei der Antikörper oder das Antigen-bindende
Fragment desselben ein monoklonaler Antikörper oder ein Antigen-bindendes Fragment desselben sind.

47. Verfahren zum Nachweis eines ausgewählten MAdCAM von Primaten in einer Probe in den Schritten:

- (a) in Kontakt Beringen einer Probe mit einem Antikörper oder Antigen-bindenden Fragment desselben nach
Anspruch 24 unter Bedingungen, die für eine spezifische Anbindung des Antikörpers oder Antigen-bindenden
Fragments desselben geeignet sind; und
- (b) Nachweis der Bildung eines Komplexes zwischen dem Antikörper oder Fragment und MAdCAM.

48. Verfahren zum Nachweis oder zur Identifizierung eines Liganden von oder eines an MAdCAM von Primaten bin-
denden Stoffes in den Schritten: Zusammenbringen eines zu untersuchenden Stoffes mit einem isolierten Protein
nach Anspruch 7 oder einer Wirtszelle nach Anspruch 12 unter Bedingungen, die für die Anbindung des Liganden
geeignet sind und Nachweisen oder Messen der Bildung eines Komplexes zwischen dem Stoff und dem Protein.

49. Verfahren zum Nachweis eines Inhibitors der Bindung eines natürlich vorkommenden MAdCAM von Primaten an
einen Liganden desselben in den Schritten:

- (a) Zusammenbringen eines zu untersuchenden Stoffes mit einem Liganden des MAdCAM von Primaten und
einer Zusammensetzung, welche ein isoliertes Protein nach Anspruch 7 oder eine Wirtszelle nach Anspruch
12 umfasst, unter Bedingungen, die zur Anbindung des Liganden geeignet sind, und
- (b) Nachweisen oder Messen der Bindung zwischen dem Protein oder der Wirtszelle und dem Liganden, wobei
eine im Vergleich mit einer geeigneten Kontrolle geringere Bindung anzeigt, dass der Stoff ein Inhibitor ist.

50. Verfahren zum Nachweis eines Inhibitors der Bindung eines natürlich vorkommenden MAdCAM von Primaten an
einen Liganden desselben in den Schritten:

- (a) Zusammenbringen eines zu untersuchenden Stoffes mit einem Liganden des MAdCAM von Primaten und
einem Immunglobulin-Hybrid nach Anspruch 18 oder 19 oder einem Fusionsprotein nach jedem der Ansprüche

14 bis 17 unter Bedingungen, die zur Bindung eines Liganden an ein natürlich vorkommendes MAdCAM von Primaten geeignet sind; und

(b) Nachweisen oder Messen der Bindung zwischen dem Immunglobulin-Hybrid oder Fusionsprotein und dem Liganden, wobei eine im Vergleich mit einer geeigneten Kontrolle geringere Bindung anzeigt, dass der Stoff ein Inhibitor ist.

51. Verfahren nach Anspruch 50, in welchem der Ligand $\alpha 4\beta 7$ -Integrin ist und ein zu untersuchender Stoff, eine $\alpha 4\beta 7$ -Integrin exprimierende Zelle und ein Immunglobulin-Hybrid nach Anspruch 18 oder ein Fusionsprotein nach Anspruch 14 oder 15 zusammengebracht werden.

52. Verfahren zum Nachweis eines Inhibitors der von MAdCAM vermittelten Zelladhäsion, welches die Schritte umfasst:

(a) Zusammenbringen eines zu untersuchenden Stoffes, einer Wirtszelle nach Anspruch 12 und einer zweiten $\alpha 4\beta 7$ -Integrin tragenden Zelle unter Bedingungen, die für die Adhäsion der Wirtszelle an die zweite Zelle geeignet sind; und

(b) Nachweisen oder Messen der Adhäsion zwischen der Wirtszelle und der zweiten Zelle, wobei eine im Vergleich mit einer geeigneten Kontrolle geringere Adhäsion anzeigt, dass der Stoff ein Inhibitor ist.

53. Verfahren nach jedem der Ansprüche 48 bis 52, in welchem der Stoff ein Antikörper oder ein Antigen-bindendes Fragment desselben ist.

54. Isolierte Nucleinsäure nach Anspruch 4, wobei die Nucleinsäure funktionsfähig an eine Kontrollsequenz für die Expression gebunden ist.

55. Verwendung nach jedem der Ansprüche 26, 31, 34 und 41, wobei die Krankheit eine Mastitis ist.

56. Verwendung nach jedem der Ansprüche 26, 31, 34 und 41, wobei die Krankheit eine Cholecystitis, Cholangitis oder Pericholangitis ist.

57. Verwendung nach jedem der Ansprüche 26, 31, 34 und 41, wobei die Krankheit eine chronische Bronchitis, eine chronische Sinusitis oder Asthma ist.

58. Verwendung nach jedem der Ansprüche 26, 31, 34 und 41, wobei die Krankheit eine Transplantat-Wirt-Reaktion ist.

59. Verwendung nach jedem der Ansprüche 26, 31, 34 und 41, wobei der Antikörper oder das Antigen-bindende Fragment desselben ausgewählt ist aus der Gruppe chimärer Antikörper, chimäres Antigen-bindendes Fragment, humanisierter Antikörper und humanisiertes Antigen-bindendes Fragment.

Revendications

1. Acide nucléique isolé codant :

(a) une MAdCAM naturellement présente chez les primates, choisie parmi le groupe constitué de la protéine représentée sur la figure 1 (SEQ ID NO: 2), la protéine représentée sur la figure 2 (SEQ ID NO: 4), la protéine représentée sur la figure 3 (SEQ ID NO: 6), et la forme mature de l'une quelconque des précédentes ; ou

(b) une variante fonctionnelle de celle-ci qui se lie à l'intégrine $\alpha 4\beta 7$; ou

(c) un fragment fonctionnel d'une MAdCAM naturellement présente chez le primate, qui se lie à l'intégrine $\alpha 4\beta 7$, et comprend au moins un domaine de type immunoglobuline, dans lequel la séquence d'acides aminés de ladite variante fonctionnelle est au moins similaire à 75 % à la séquence d'une protéine représentée sur la figure 1 (SEQ ID NO: 2), la figure 2 (SEQ ID NO: 4) ou la figure 3 (SEQ ID NO: 6), et la similarité de séquence des acides aminés est déterminée par la méthode Clustal avec la matrice de pondération des résidus PAM250, en utilisant une pénalité d'insertion de 10, une pénalité de longueur d'insertion de 10 et des paramètres d'alignement de paires de ktuple = 1, pénalité d'insertion = 3, fenêtre = 4 et diagonales sauvegardées = 5.

2. Acide nucléique isolé selon la revendication 1, dans lequel l'acide nucléique isolé est recombinant et/ou essentiellement pur.

3. Acide nucléique isolé selon la revendication 1 ou la revendication 2, dans lequel,

(a) ledit acide nucléique s'hybride dans des conditions de haute stringence avec un deuxième acide nucléique, le deuxième acide nucléique ayant une séquence de nucléotides telle que représentée sur la figure 1 (SEQ ID NO: 1), la figure 2 (SEQ ID NO: 3) ou la figure 3 (SEQ ID NO: 5), ou une séquence qui est le complément du cadre ouvert de lecture de l'une quelconque des précédentes ; ou

(b) ledit acide nucléique code le polypeptide représenté sur la figure 1 (SEQ ID NO: 2), le polypeptide représenté sur la figure 2 (SEQ ID NO: 4), le polypeptide représenté sur la figure 3 (SEQ ID NO: 6), ou les protéines matures correspondantes.

4. Acide nucléique isolé selon la revendication 3, dans lequel ledit acide nucléique comporte une séquence de nucléotides choisie parmi le groupe consistant en une séquence de nucléotides telle que représentée sur la figure 1 (SEQ ID NO: 1), une séquence de nucléotides telle que représentée sur la figure 2 (SEQ ID NO: 3), une séquence de nucléotides telle que représentée sur la figure 3 (SEQ ID NO: 5), et une partie de l'une quelconque des précédentes comprenant la séquence codante.

5. Construction d'acide nucléique recombinant isolé, comprenant un acide nucléique selon la revendication 1.

6. La construction d'acide nucléique recombinant isolé, selon la revendication 5,

(a) dans lequel l'acide nucléique recombinant est lié de façon opérationnelle à une séquence de contrôle de l'expression ; ou

(b) comprenant un acide nucléique, dans lequel ledit acide nucléique code un polypeptide ayant une séquence d'acides aminés telle qu'exposée sur la figure 1 (SEQ ID NO: 2), la figure 2 (SEQ ID NO: 4) ou la figure 3 (SEQ ID NO: 6) ; ou

(c) ledit acide nucléique s'hybride dans des conditions de haute stringence avec un deuxième acide nucléique, le deuxième acide nucléique ayant une séquence de nucléotides telle que représentée sur la figure 1 (SEQ ID NO: 1), la figure 2 (SEQ ID NO: 3), la figure 3 (SEQ ID NO: 5), ou une séquence qui est le complément du cadre ouvert de lecture de l'une quelconque des précédentes.

7. Protéine isolée qui possède la séquence d'acides aminés :

(a) d'une MAdCAM naturellement présente chez les primates, choisie parmi le groupe constitué de la protéine représentée sur la figure 1 (SEQ ID NO: 2), la protéine représentée sur la figure 2 (SEQ ID NO: 4), la protéine représentée sur la figure 3 (SEQ ID NO: 6), et la forme mature de l'une quelconque des précédentes, ou

(b) d'une variante fonctionnelle de celle-ci qui se lie à l'intégrine $\alpha 4 \beta 7$; ou

(c) d'un fragment fonctionnel d'une MAdCAM naturellement présente chez le primate, qui se lie à l'intégrine $\alpha 4 \beta 7$, et comprend au moins un domaine de type immunoglobuline, dans lequel la séquence des acides aminés de ladite variante fonctionnelle est au moins similaire à 75 % à la séquence d'une protéine représentée sur la figure 1 (SEQ ID NO: 2), la figure 2 (SEQ ID NO: 4) ou la figure 3 (SEQ ID NO: 6), et la similarité de séquence d'acides aminés est déterminée par la méthode Clustal avec la matrice de pondération des résidus PAM250, en utilisant une pénalité d'insertion de 10, une pénalité de longueur d'insertion de 10 et des paramètres d'alignement de paires de ktuple = 1, pénalité d'insertion = 3, fenêtre = 4 et diagonales sauvegardées = 5.

8. Protéine isolée selon la revendication 7, dans laquelle,

(a) la MAdCAM de primate est une MAdCAM humaine, codée par un acide nucléique qui s'hybride dans des conditions de haute stringence à un deuxième acide nucléique, le deuxième acide nucléique ayant une séquence de nucléotides telle que représentée sur la figure 1 (SEQ ID NO: 1), la figure 2 (SEQ ID NO: 3), la figure 3 (SEQ ID NO: 5), ou une séquence qui est le complément du cadre ouvert de lecture de l'une quelconque des précédentes ; ou

(b) la MAdCAM de primate est une MAdCAM humaine telle que représentée sur la figure 1 (SEQ ID NO: 2), la figure 2 (SEQ ID NO: 4), ou la protéine mature correspondante de l'une ou l'autre des précédentes ; ou

(c) la MAdCAM de primate est une MAdCAM de macaque comme représenté sur la figure 3 (SEQ ID NO: 6), ou la protéine mature correspondante.

9. MAdCAM naturellement présente chez le primate, isolée, possédant une ou plusieurs fonctions choisies parmi le groupe constitué d'une liaison à l'intégrine $\alpha 4 \beta 7$ et d'une médiation de l'adhésion cellulaire.

10. MAdCAM de primate, isolée, selon la revendication 9, dans laquelle l'adhésion cellulaire est dépendante de l'intégrine $\alpha 4\beta 7$.

11. MAdCAM de primate, isolée, selon la revendication 9 ou la revendication 10, dans laquelle ladite liaison est sélective pour $\alpha 4\beta 7$.

12. Cellule hôte comprenant un acide nucléique recombinant qui code :

(a) une MAdCAM naturellement présente chez le primate, choisie parmi le groupe constitué de la protéine représentée sur la figure 1 (SEQ ID NO: 2), la protéine représentée sur la figure 2 (SEQ ID NO: 4), la protéine représentée sur la figure 3 (SEQ ID NO: 6), et la forme mature d'une quelconque des précédentes ; ou

(b) une variante fonctionnelle de celle-ci qui se lie à l'intégrine $\alpha 4\beta 7$; ou

(c) un fragment fonctionnel d'une MAdCAM naturellement présente chez le primate, qui se lie à l'intégrine $\alpha 4\beta 7$ et comprend au moins un domaine de type immunoglobuline, dans lequel la séquence d'acides aminés de ladite variante fonctionnelle est au moins similaire à 75 % à la séquence d'une protéine représentée sur la figure 1 (SEQ ID NO: 2), la figure 2 (SEQ ID NO: 4) ou la figure 3 (SEQ ID NO: 6), et la similarité de séquence des acides aminés est déterminée par la méthode Clustal avec la matrice de pondération des résidus PAM250, en utilisant une pénalité d'insertion de 10, une pénalité de longueur d'insertion de 10 et des paramètres d'alignement de paires de ktuple = 1, pénalité d'insertion = 3, fenêtre = 4 et diagonales sauvegardées = 5..

13. Cellule hôte selon la revendication 12, dans laquelle ledit acide nucléique recombinant est lié de façon opérationnelle à une séquence de contrôle de l'expression, au moyen de quoi ladite MAdCAM naturellement présente chez le primate, ou ladite variante fonctionnelle qui se lie à l'intégrine $\alpha 4\beta 7$ ou ledit fragment fonctionnel d'une MAdCAM naturellement présente chez le primate, qui se lie à l'intégrine $\alpha 4\beta 7$, est exprimée lorsque la cellule hôte est maintenue dans des conditions appropriées pour l'expression.

14. Protéine de fusion comprenant:

(a) une MAdCAM naturellement présente chez le primate, choisie parmi le groupe constitué de la protéine représentée sur la figure 1 (SEQ ID NO: 2), la protéine représentée sur la figure 2 (SEQ ID NO: 4), la protéine représentée sur la figure 3 (SEQ ID NO: 6), et la forme mature de l'une quelconque des précédentes, ou

(b) une variante fonctionnelle de celle-ci qui se lie à l'intégrine $\alpha 4\beta 7$; ou

(c) un fragment fonctionnel d'une MAdCAM naturellement présente chez les primates, qui se lie à l'intégrine $\alpha 4\beta 7$ et comprend au moins un domaine de type immunoglobuline, dans lequel la séquence d'acides aminés de ladite variante fonctionnelle est au moins similaire à 75 % à la séquence d'une protéine représentée sur la figure 1 (SEQ ID NO: 2), la figure 2 (SEQ ID NO: 4) ou la figure 3 (SEQ ID NO: 6), et la similarité de séquence d'acides aminés est déterminée par la méthode Clustal avec la matrice de pondération des résidus PAM250, en utilisant une pénalité d'insertion de 10, une pénalité de longueur d'insertion de 10 et des paramètres d'alignement de paires de ktuple = 1, pénalité d'insertion = 3, fenêtre = 4 et diagonales sauvegardées = 5..

15. Protéine de fusion selon la revendication 14, comprenant un premier fragment et un deuxième fragment, dans laquelle ledit premier fragment possède la séquence d'acides aminés d'une MAdCAM naturellement présente chez le primate, ou ladite variante fonctionnelle de celle-ci ou ledit fragment fonctionnel, et ledit deuxième fragment est au moins une partie d'une chaîne d'immunoglobuline ou une variante de celle-ci.

16. Protéine de fusion selon la revendication 15, dans laquelle,

(a) ledit premier fragment est joint à son extrémité C-terminale à l'extrémité N-terminale du deuxième fragment ; ou

(b) le premier fragment est choisi parmi le groupe constitué d'un fragment fonctionnel d'une MAdCAM humaine contenant l'intégralité du domaine extracellulaire, et un fragment fonctionnel d'une MAdCAM humaine contenant deux domaines immunoglobuline N-terminaux ; ou

(c) le deuxième fragment est au moins une partie d'une région constante à chaîne lourde d'immunoglobuline ou une variante fonctionnelle de celle-ci.

17. Protéine de fusion selon la revendication 16, dans laquelle la chaîne lourde d'immunoglobuline est de la classe IgG ; et/ou le deuxième fragment comprend des domaines charnières, CH2 et CH3 d'une chaîne lourde d'immunoglobuline.

18. Immunoglobuline hybride comprenant une protéine hybride selon l'une quelconque des revendications 14 à 17.
19. Immunoglobuline hybride selon la revendication 18, dans laquelle ladite immunoglobuline hybride est un homodimère.
20. Construction d'acide nucléique recombinante, comprenant un acide nucléique contenant une séquence codante qui code une protéine de fusion selon l'une quelconque des revendications 14 à 17, dans lequel la séquence codante est éventuellement liée de façon opérationnelle à une séquence de contrôle de l'expression.
21. Procédé de production d'une protéine selon la revendication 7, comprenant le maintien d'une cellule hôte, comprenant un acide nucléique recombinant codant ladite protéine, dans des conditions appropriées pour l'expression de l'acide nucléique, au moyen de quoi ladite protéine est produite.
22. Procédé selon la revendication 21, comprenant en outre :
 - (a) l'introduction dans une cellule hôte d'une construction d'acide nucléique, comprenant un acide nucléique qui code ladite protéine, au moyen de quoi est produite une cellule hôte recombinante, comportant ladite séquence de codage liée de façon opérationnelle à au moins une séquence de contrôle de l'expression ; et
 - (b) le maintien des cellules hôtes produites à l'étape (a) dans un milieu approprié dans des conditions au moyen desquelles l'acide nucléique est exprimé.
23. Procédé selon la revendication 21 ou la revendication 22, comprenant en outre l'étape d'isolement de ladite protéine.
24. Anticorps ou fragment de liaison à l'antigène qui lie une MAdCAM naturellement présente chez le primate, une variante fonctionnelle d'une MAdCAM naturellement présente chez le primate, qui se lie à une intégrine $\alpha 4 \beta 7$ et/ou un fragment fonctionnel d'une MAdCAM naturellement présente chez les primates, qui se lie à l'intégrine $\alpha 4 \beta 7$, et comprend au moins un domaine de type immunoglobuline, comme défini selon la revendication 7.
25. Anticorps ou fragment de liaison à l'antigène selon la revendication 24, pour une utilisation en thérapie ou en diagnostic.
26. Utilisation d'un anticorps ou fragment de liaison à l'antigène selon la revendication 24, pour la fabrication d'un médicament pour traiter une maladie choisie parmi le groupe constitué de la pancréatite, du diabète sucré insulino-dépendant, de la mastite, de la cholécystite, de l'angiocholite, de la périangiocholite, de la bronchite chronique, de la sinusite chronique, de l'asthme et de la réaction du greffar contre l'hôte.
27. Utilisation d'un anticorps ou d'un fragment de liaison antigène selon la revendication 24, pour la fabrication d'un médicament pour traiter une maladie inflammatoire de l'intestin.
28. Anticorps ou fragment de liaison à l'antigène selon la revendication 24 ou la revendication 25, ou utilisation selon la revendication 27, dans lequel,
 - (a) ledit anticorps ou fragment de liaison antigène peut inhiber l'une ou les deux de la fonction de liaison ou de la fonction moléculaire d'adhésion cellulaire d'une MAdCAM naturellement présente chez les primates ; ou
 - (b) ledit anticorps ou fragment de liaison à l'antigène peut de façon sélective inhiber l'adhésion dépendante de $\alpha 4 \beta 7$; ou
 - (c) ledit anticorps ou fragment de liaison antigène peut inhiber la liaison d'une MAdCAM naturellement présente chez les primates à l'intégrine $\alpha 4 \beta 7$.
29. Anticorps ou fragment de liaison à l'antigène selon la revendication 24, la revendication 25 ou la revendication 28, ou utilisation selon la revendication 27 ou la revendication 28, dans lequel ledit anticorps ou fragment de liaison antigène est un anticorps monoclonal ou un fragment de liaison antigène de celui-ci.
30. Anticorps ou fragment de liaison à l'antigène selon la revendication 24, 25, 28 ou 29, ou utilisation selon l'une quelconque des revendications 27 à 29, dans lequel ladite MAdCAM naturellement présente chez le primate est une MAdCAM naturellement présente chez l'homme.
31. Utilisation d'une MAdCAM de primate, d'une variante fonctionnelle de celle-ci qui se lie à l'intégrine $\alpha 4 \beta 7$, ou d'une

immunoglobuline hybride comprenant une MAdCAM de primate ou variante de liaison à l'intégrine $\alpha 4\beta 7$ de celle-ci, pour la fabrication d'un médicament pour traiter une maladie choisie parmi le groupe constitué de la pancréatite, du diabète sucré insulino-dépendant, de la mastite, de la cholécystite, de l'angiocholite, de la périangiocholite, de la bronchite chronique, de la sinusite chronique, de l'asthme et de la réaction de greffon contre l'hôte,

dans laquelle ladite MAdCAM de primate est une protéine ayant la séquence d'acides aminés d'une MAdCAM naturellement présente chez le primate, choisie parmi le groupe constitué de la protéine représentée sur la figure 1 (SEQ ID NO: 2), la protéine représentée sur la figure 2 (SEQ ID NO: 4), la protéine représentée sur la figure 3 (SEQ ID NO: 6), et la forme mature de l'une quelconque des précédentes, ou une partie fonctionnelle de celle-ci qui se lie à l'intégrine $\alpha 4\beta 7$, ladite partie fonctionnelle comprend au moins un domaine de type immunoglobuline, et dans laquelle la séquence d'acides aminés de ladite variante fonctionnelle est au moins similaire à 75 % à la séquence d'une protéine représentée sur la figure 1 (SEQ ID NO: 2), la figure 2 (SEQ ID NO: 4) ou la figure 3 (SEQ ID NO: 6), et la similarité de séquence des acides aminés est déterminée par la méthode de Clustal avec la matrice de pondération des résidus PAM250, en utilisant une pénalité d'insertion de 10, une pénalité de longueur d'insertion de 10 et des paramètres d'alignement de paires de ktuple = 1, pénalité d'insertion = 3, fenêtre = 4 et diagonales sauvegardées = 5.

- 32.** Utilisation d'une MAdCAM de primate, d'une variante fonctionnelle de celle-ci qui se lie à l'intégrine $\alpha 4\beta 7$, ou d'une immunoglobuline hybride comprenant une MAdCAM de primate ou une variante de liaison à l'intégrine $\alpha 4\beta 7$ de celle-ci, pour la fabrication d'un médicament pour traiter une maladie inflammatoire de l'intestin, dans laquelle ladite MAdCAM de primate est une protéine ayant la séquence d'acides aminés d'une MAdCAM naturellement présente chez le primate, choisie parmi le groupe constitué de la protéine représentée sur la figure 1 (SEQ ID NO: 2), la protéine représentée sur la figure 2 (SEQ ID NO: 4), la protéine représentée sur la figure 3 (SEQ ID NO: 6), et la forme mature de l'une quelconque des précédentes, ou une partie fonctionnelle de celle-ci qui se lie à l'intégrine $\alpha 4\beta 7$, ladite partie fonctionnelle comprend au moins un domaine de type immunoglobuline, et dans lequel la séquence d'acides aminés de ladite variante fonctionnelle est au moins similaire à 75 % à la séquence d'une protéine représentée sur la figure 1 (SEQ ID NO: 2), la figure 2 (SEQ ID NO: 4) ou la figure 3 (SEQ ID NO: 6), et la similarité de séquence des acides aminés est déterminée par la méthode Clustal avec la matrice de pondération des résidus PAM250, en utilisant une pénalité d'insertion de 10, une pénalité de longueur d'insertion de 10 et des paramètres d'alignement de paires de ktuple = 1, pénalité d'insertion = 3, fenêtre = 4 et diagonales sauvegardées = 5.

- 33.** Utilisation selon la revendication 32, dans laquelle,

(a) la MAdCAM de primate, une variante fonctionnelle ou une immunoglobuline hybride peut inhiber l'interaction d'une MAdCAM de primate avec une intégrine $\alpha 4\beta 7$; ou

(b) ladite immunoglobuline hybride contient une protéine de fusion, comprenant un premier fragment et un deuxième fragment, dans laquelle ledit premier fragment est une MAdCAM de primate, et ledit deuxième fragment est au moins une partie d'une chaîne d'immunoglobuline.

- 34.** Utilisation d'un anticorps ou d'un fragment de liaison à l'antigène de celui-ci qui lie la chaîne $\beta 7$ de l'intégrine $\alpha 4\beta 7$ et inhibe la liaison de MAdCAM de primate à l'intégrine $\alpha 4\beta 7$, pour la fabrication d'un médicament pour traiter une maladie choisie 5 parmi le groupe constitué de la pancréatite, du diabète sucré insulino-dépendant, de la mastite, de la cholécystite, de l'angiocholite, de la périangiocholite, de la bronchite chronique, de la sinusite chronique, de l'asthme et de la réaction du greffon contre l'hôte, dans lequel ladite MAdCAM de primate est :

(a) une MAdCAM naturellement présente chez les primates, choisie parmi le groupe constitué de la protéine représentée sur la figure 1 (SEQ ID NO: 2), la protéine représentée sur la figure 2 (SEQ ID NO: 4), la protéine représentée sur la figure 3 (SEQ ID NO: 6), et la forme mature de l'une quelconque des précédentes, ou
(b) une variante fonctionnelle de celle-ci qui se lie à l'intégrine $\alpha 4\beta 7$, et

dans laquelle la séquence d'acides aminés de ladite variante fonctionnelle est au moins similaire à 75 % à la séquence d'une protéine représentée sur la figure 1 (SEQ ID NO: 2), la figure 2 (SEQ ID NO: 4) ou la figure 3 (SEQ ID NO: 6), et la similarité de séquence d'acides aminés est déterminée par la méthode Clustal avec la matrice de pondération des résidus PAM250, en utilisant une pénalité d'insertion de 10, une pénalité de longueur d'insertion de 10 et des paramètres d'alignement de paires de ktuple = 1, pénalité d'insertion = 3, fenêtre = 4 et diagonales sauvegardées = 5.

- 35.** Utilisation d'un anticorps ou d'un fragment de liaison à l'antigène de celui-ci qui lie la chaîne $\beta 7$ de l'intégrine $\alpha 4\beta 7$ et inhibe la liaison de la MAdCAM à l'intégrine $\alpha 4\beta 7$, pour la fabrication d'un médicament pour traiter une maladie

inflammatoire de l' intestin.

36. Utilisation selon la revendication 35, dans laquelle un anticorps ou un fragment de liaison à l'antigène de celui-ci inhibe l'adhésion des leucocytes exprimant une intégrine contenant la chaîne $\beta 7$, à un endothélium exprimant une MAdCAM.

37. Utilisation selon la revendication 35 ou 36, dans laquelle,

(a) on emploie plus d'un anticorps ou fragment de liaison à l'antigène de celui-ci qui inhibe la liaison des leucocytes à la MAdCAM endothéliale ; ou

(b) on emploie plus d'un anticorps ou fragment de liaison antigène de celui-ci qui inhibe la liaison des leucocytes aux ligands endothéliaux, et au moins un anticorps ou un fragment inhibe la liaison des leucocytes à un ligand endothélial autre que la MAdCAM.

38. Utilisation selon la revendication 35, dans laquelle un anticorps ou un fragment de liaison à l'antigène inhibe la liaison des leucocytes à la MAdCAM endothéliale.

39. Utilisation selon l'une quelconque des revendications 27 à 30, dans laquelle l'anticorps ou le fragment de liaison à l'antigène de celui-ci est choisi parmi le groupe constitué d'un anticorps chimère, un fragment de liaison antigène chimère, un anticorps humanisé, et un fragment de liaison antigène humanisé.

40. Utilisation selon l'une quelconque des revendications 27 à 30, dans laquelle la maladie est une colite ulcéreuse, la maladie de Crohn, la maladie coeliaque, l'entéropathie associée à des arthropathies séronégatives, la colite microscopique ou collagène, une gastroentérite éosinophile ou une pochite.

41. Utilisation d'un anticorps ou d'un fragment de liaison à l'antigène de celui-ci qui se lie à l'intégrine $\alpha 4\beta 7$, pour la fabrication d'un médicament pour traiter une maladie choisie parmi le groupe constitué de la pancréatite, du diabète sucré insulino-dépendant, de la mastite, de la cholécystite, de l'angiocholite, de la périangiocholite, de la bronchite chronique, de la sinusite chronique, de l'asthme et de la réaction du greffon contre l'hôte, dans laquelle ledit anticorps ou fragment de liaison antigène possède la spécificité épitopique de l'anticorps monoclonal ACT-1.

42. Utilisation selon l'une quelconque des revendications 26, 31, 34 et 41, dans laquelle ladite maladie est une pancréatite ou un diabète sucré insulino-dépendant.

43. Utilisation d'un anticorps ou d'un fragment de liaison à l'antigène de celui-ci, qui se lie à l'intégrine $\alpha 4\beta 7$, pour la fabrication d'un médicament pour traiter une maladie inflammatoire de l'intestin chez un humain, dans laquelle ledit anticorps ou fragment de liaison à l'antigène possède la spécificité épitopique de l'anticorps monoclonal ACT-1.

44. Utilisation selon l'une quelconque des revendications 35 à 38 et 43, dans laquelle ladite maladie inflammatoire de l'intestin est choisie parmi le groupe constitué de la colite ulcéreuse, la maladie de Crohn, la maladie coeliaque, l'entéropathie associée aux arthropathies séronégatives, la colite microscopique ou collagène, la gastroentérite éosinophile et la pochite.

45. Utilisation selon l'une quelconque des revendications 35 à 38, 43 et 44, dans laquelle l'anticorps ou le fragment de liaison antigène de celui-ci est choisi parmi le groupe constitué d'un anticorps chimère, d'un fragment de liaison à l'antigène chimère, d'un anticorps humanisé, et d'un fragment de liaison à l'antigène humanisé.

46. Utilisation selon l'une quelconque des revendications 35 à 38, 43 et 44, dans laquelle l'anticorps ou le fragment de liaison antigène de celui-ci est un anticorps monoclonal ou un fragment de liaison à l'antigène de celui-ci.

47. Procédé de détection d'une MAdCAM de primate choisie dans un échantillon, comprenant :

(a) la mise en contact d'un échantillon avec un anticorps ou un fragment de liaison à l'antigène de celui-ci selon la revendication 24, dans des conditions appropriées pour une liaison spécifique dudit anticorps ou fragment de celui-ci ; et

(b) la détection de la formation d'un complexe entre ledit anticorps ou fragment et une MAdCAM.

48. Procédé de détection ou d'identification d'un ligand ou d'un agent qui lie une MAdCAM de primate, comprenant la

combinaison d'un agent à tester, avec une protéine isolée selon la revendication 7, ou une cellule hôte selon la revendication 12, dans des conditions appropriées pour la liaison d'un ligand à celle-ci, et la détection ou la mesure de la formation d'un complexe entre ledit agent et ladite protéine.

5 **49.** Procédé de détection d'un inhibiteur de la liaison d'une MAdCAM naturellement présente chez les primates, à un ligand de celle-ci, comprenant :

- 10 (a) la combinaison d'un agent à tester, avec un ligand de ladite MAdCAM de primate et une composition comprenant une protéine isolée selon la revendication 7, ou une cellule hôte selon la revendication 12, dans des conditions appropriées pour la liaison du ligand à celle-ci ; et
(b) la détection ou la mesure de la liaison entre ladite protéine ou la cellule hôte et le ligand, au moyen de quoi une liaison diminuée, par comparaison à un témoin approprié, indique que l'agent est un inhibiteur.

15 **50.** Procédé de détection d'un inhibiteur de la liaison d'une MAdCAM naturellement présente chez les primates, à un ligand de celle-ci, comprenant :

- 20 (a) la combinaison d'un agent à tester, avec un ligand de ladite MAdCAM de primate et une immunoglobuline hybride selon la revendication 18 ou 19, ou une protéine de fusion selon l'une quelconque des revendications 14 à 17, dans des conditions appropriées pour la liaison du ligand à une MAdCAM naturellement présente chez le primate ; et
(b) la détection ou la mesure de la liaison entre ladite immunoglobuline hybride ou protéine de fusion et ledit ligand, au moyen de quoi une liaison diminuée, par comparaison à un témoin approprié, indique que l'agent est un inhibiteur.

25 **51.** Procédé selon la revendication 50, dans lequel ledit ligand est l'intégrine $\alpha 4\beta 7$, et un agent à tester, une cellule exprimant l'intégrine $\alpha 4\beta 7$; et une immunoglobuline hybride selon la revendication 18, ou une protéine de fusion selon la revendication 14 ou la revendication 15, sont combinées.

30 **52.** Procédé de détection d'un inhibiteur de l'adhésion cellulaire à médiation MAdCAM, comprenant :

- (a) la combinaison d'un agent à tester, d'une cellule hôte selon la revendication 12, et d'une deuxième cellule portant une intégrine $\alpha 4\beta 7$, dans des conditions appropriées pour l'adhésion de ladite cellule hôte à ladite deuxième cellule ; et
35 (b) la détection ou la mesure de l'adhésion entre lesdites cellule hôte et deuxième cellule, au moyen de quoi une adhésion diminuée, par comparaison à un témoin approprié, indique que l'agent est un inhibiteur.

53. Procédé selon l'une quelconque des revendications 48 à 52, dans lequel l'agent est un anticorps ou un fragment de liaison antigène de celui-ci.

40 **54.** Acide nucléique isolé selon la revendication 4, dans lequel l'acide nucléique est lié de façon opérationnelle à une séquence de contrôle de l'expression.

55. Utilisation selon l'une quelconque des revendications 26, 31, 34 et 41, dans laquelle ladite maladie est une mastite.

45 **56.** Utilisation selon l'une quelconque des revendications 26, 31, 34 et 41, dans laquelle ladite maladie est une cholestite, une angiocholite ou une périangiocholite.

57. Utilisation selon l'une quelconque des revendications 26, 31, 34 et 41, dans laquelle ladite maladie est une bronchite chronique, une sinusite chronique ou l'asthme.

50 **58.** Utilisation selon l'une quelconque des revendications 26, 31, 34 et 41, dans laquelle ladite maladie est une réaction du greffon contre l'hôte.

55 **59.** Utilisation selon l'une quelconque des revendications 26, 34 et 41, dans laquelle l'anticorps ou le fragment de liaison à l'antigène de celui-ci est choisi parmi le groupe constitué d'un anticorps chimère, d'un fragment de liaison à l'antigène chimère, d'un anticorps humanisé, et d'un fragment de liaison à l'antigène humanisé.

ATGGATTTCGGACTGGCCCTCCTGCTGGCGGGGCTTCTGGGGCTCCTCCTCGGCCAGTCCCTCCAGGTGAAGCCCTGCA 80
 M D F G L A L L L A G L L G L L L G O S L O V K P L O
 GGTGGAGCCCCGGAGCCGGTGGTGGCCGTGGCCCTGGGGCGCTCGCGCCAGCTCACCTGCCGCCTGGCCTGCGGGGACC 160
 V E P P E P V V A V A L G A S R O L T **C** R L A **C** A D
 GCGGGGCTCGGTGCAGTGGCGGGGCTGGACACCAAGCCTGGGCGCGGTGCAGTCGGACACGGGGCCGACGCTCCTCACC 240
 R G A S V O W R G L D T S L G A V O S D T G R S V L T
 GTGGCAACGCCTCGCTGTGCGGGGCGGGGACCCGCGTGTGCGTGGGCTCCTGCGGGGGCCGCACCTTCCAGCACACCGT 320
 V R **N A S** L S A A G T R V **C** V G S **C** G G R T F O H T V
 GCAGCTCCTTGTGTACGCCTTCCCGGACCACTGACCGTCTCCCCAGCAGCCCTGGTGCCTGGTGACCCGGAGGTGGCCT 400
 O L L V Y A F P D O L T V S P A A L V P G D P E V A
 GTACGGCCCAAAAGTCACGCCCCGTGGACCCCAACGCGCTCTCCTTCTCCTGCTCGTGGGGGGCCAGGAACCTGGAGGGG 480
C T A H K V T P V D P N A L S F S L L V G G O E L E G
 GCGCAAGCCCTGGCCCCGGAGGTGCAGGAGGAGGAGGAGGAGCCCGAGGGGACGAGGACGTGCTGTTTCAGGGTGACAGA 560
 A O A L G P E V O E E E E P O G D E D V L F R V T E
 GCGTGGCGGCTGCCGCCCTGGGGACCCCTGTCCCGCCCGCCCTCTACTGCCAGGCCACGATGAGGCTGCCTGGCTTGG 640
 R W R L P P L G T P V P P A L Y **C** O A T M R L P G L
 AGCTCAGCCACCGCCAGGCCATCCCGTCTGCAAGCCCCGACCTCCCCGGAGCCTCCCGACACCACCTCCCGGAGCCT 720
 E L S H R O A I P V L H S P T S P E P P D T T S P E P
 CCAACACCACTCCCGGAGTCTCCCGACACCACCTCCCGGAGTCTCCCGACACCACCTCCAGGAGCCTCCCGACAC 800
 P **N T T** S P E S P D T T S P E S P D T T S O E P P D T
 CACCTCCCGAGGAGCTCCCGACACCACCTCCCGAGGAGCCTCCCGACACCACCTCCCGGAGCCTCCCGACAGACCTCCC 880
 T S O E P P D T T S O E P P D T T S P E P P D K T S
 CGGAGCCCGCCCCCAGCAGGGCTCCACACACACCCCCAGGAGCCAGGCTCCACCAGGACTCGCCGCCCTGAGATCTCC 960
 P E P A P O O G S T H T P R S P G S T R T R R P E I S
 CAGGCTGGGCCCCAGCAGGGAGAAGTGATCCCAACAGGCTCGTCCAAACCTGCGGGTGACCAGCTGCCCGCGGCTCTGTG 1040
 O A G F T O G E V I P T G S S K P A G D O L P A A L W
 GACCAGCAGTGGGCTGCTGGGACTGCTGCTCTGGCCTTGGCCACGTATCACCTCTGGAACGCTGCCGGCACCTGGCTG 1120
 T S S A V L G L L L L A L P T Y H L W K R C R H L A
 ASSACGACACCCACCCACCACTTCTGTAGGCTTCTGCCCCAGGTGTCGGCCTGGGCTGGGTTAAGGGGACCGGCCAG 1200
 E D D T H P P A S L R L L P O V S A W A G L R G T G O
 GTGGGATCAGCCCTCCTGAGTGGCCAGCCTTTCCCCCTGTGAAAGCAAAATAGCTTGACCCCTTCAAGTTGAGAACT 1280
 V G I S P S
 GGTACGGGCAAACTGCCTCCCATCTACTCAAAGTCATCCCTCTGCTCACAGAGATGGATGCATGTTCTGATTGCCTCT 1360
 TTGGAGAAGCTCATCAGAACTCAAAAGAAGGCCACTGTTTGTCTCACCTACCCATGACCTGAAGCCCCCTCCCTGAGTGG 1440
 TCCCCACCTTCTGGACGGAACCACTACTTTTACATACATTGATTCATGTCTCACGTCTCCCTAAAAATGCGTAAGAC 1520
 CAAGTGTGCCCTGACCACCTGGGCCCCCTGTGCTCAGGACCTCCTGAGGCTTTGGCAAATAAACCTCTAAATGATAA 1600
 AAAAAAAAAAAAAAAAAAAAAA 1624

FIGURE 1

ATGGATTTCGGACTGGCCCTCCTGCTGGCGGGGCTTCTGGGGCTCCTCTCGGCCAGTCCAGCTCCAGGTGAAGCCCTGCA 80
M D F G L A L L L A G L L G L L L G Q S L Q V K P L Q

GGTGGAGCCCCCGGAGCCGGTGGTGGCCGTGGCCTTGGGCGCCTCGGCCAGCTCACCTGCCGCTGGCCTGCGCGGACC 160
V E P P E P V V A V A L G A S R Q L T C R L A C A D

GCGGGGCTCGGTGCAGTGGCGGGGCTGGACACCAGCCTGGGCGCGGTGCAGTGGACACGGGCCGAGCGTCTCTACC 240
R G A S V Q W R G L D T S L G A V Q S D T G R S V L T

GTGCGCAACGCCTCGTGTGCGGGCGGGGACCCGCGTGTGCGTGGGCTCCTGCGGGGGCCGACCTTCCAGCACACCGT 320
V R N A S L S A A G T R V C V G S C G G R T F Q H T V

GCAGCTCCTGTGTACGCTTCCCGGACCAGCTGACCGTCTCCCCAGCAGCCCTGGTGCCTGGTGACCCGGAGGTGGCCT 400
Q L L V Y A F P D Q L T V S P A A L V P G D P E V A

GTACGGCCCAAAAGTCACGCCCGTGGACCCCAACGCGCTCTCCTTCTCCTGCTCGTGGGGGCCAGGAACTGGAGGGG 480
C T A H K V T P V D P N A L S F S L L V G G Q E L E G

GCGCAAGCCCTGGGCCCCGAGGTGCAGGAGGAGGAGGAGGCCAGGGGGACGAGGACGTGCTGTTCAAGGTGACAGA 560
A O A L G P E V Q E E E E E P Q G D E D V L F R V T E

GCGCTGGCGGCTGCCGCCCTGGGGACCCCTGTCCCGCCCGCCTCTACTGCCAGGCCACGATGAGGCTGCCTGGCTTGG 640
R W R L P P L G T P V P P A L Y C Q A T M R L P G L

AGCTCAGCCACCGCCAGGCCATCCCGTCTCTGCACAGCCCGACCTCCCCGGAGCCTCCCGACACCACCTCCCCGGAGTCT 720
E L S H R O A I P V L H S P T S S P E P P D T T S P E S

CCCGACACCACCTCCCCGAGTCTCCCGACACCACCTCCCGAGGCTCCCGACACCACCTCCCCGGAGCCTCCCGACAA 800
P D T T S P E S P D T T S Q E P P D T T S P E P P D K

GACCTCCCCGGAGCCCGCCCCCAGCAGGGCTCCACACACACCCCGAGGAGCCAGGCTCCACCAGGACTCGCCGCCCTG 880
T S P E P A P Q Q G S T H T P R S P G S T R T R R P

AGATCTCCAGGCTGGGCCACGCAGGGAGAAGTGATCCCAACAGGCTCGTCCAAACCTGCGGGTGACCAGCTGCCCGCG 960
E I S Q A G P T Q G E V I P T G S S K P A G D Q L P A

GCTCTGTGGACCAGCAGTGGGTGCTGGGACTGCTGCTCTGGCCTTGCCACCTATCACCTCTGGAACGCTGCCGGCA 1040
A L W T S S A V L G L L L A L P T Y H L W K R C R H

CCTGGCTGAGGACGACACCCACCCACGCTTCTCTGAGGCTTCTGCCCCAGGTGTGGCCTGGGCTGGGTTAAGGGGGA 1120
L A E D D T H P P A S L R L L P O V S A W A G L R G

CCGSCCAGGTGGGATCAGCCCCCTCTGAGTGGCCAGCCTTCCCCCTGTGAAAGCAAAATAGCTTGGACCCCTTCAAGT 1200
T G Q V G I S P S

TGAGAACTGGTCAGGGCAAACCTGCCTCCATTCTACTCAAAGTCATCCCTCTGTTTCACAGAGATGGATGCATGTTCTGA 1280

TTGCCTCTTTGGAGAAGCTCATCAGAAACTCAAAGAAGGCCACTGTTTGTCTCACCTACCCATGACCTGAAGCCCTCC 1360

CTGAGTGGTCCCCACCTTTCTGGACGGAACACGTACTTTTACATACATTGATTTCATGTCTCACGTCTCCCTAAAAATG 1440

CGTAAGACCAAGCTGTGCCTGACCACCTGGGCCCCCTGTGTCAGGACCTCTGAGGCTTTGGCAAATAAACCTCTTAA 1520

AATGAAAAAAAAAAAAAAAAA 1539

Figure 3

AGCATGGATCGGGGCTGCCCCCTCTGCTGGGGGGCTTCTGGGGCTCCTCCAGCCGSGCTCGGGCCAGTCCCTCCAGGT 80
 M D R G L A L L L A G L L C L L O P G C G O S L Q V
 GAAGCCCCCTCCAGCTCCAGCCCCCGGAGCCGGTGGTGGCCGTGGCCCTGGGGGGCTCTCCAGCTCAGCTGCCCCCTGG 160
 K P L O V E P P F P V V A V A L G A S R Q L I E R L
 ACTGCGCGACCGGGGGCCACGGTGCAGTGGGGGGCCCTGGACACCACCTTGGGSGGGTGCAGTCCGACGCGGGCCCG 240
 D E A D R G A T V Q W R G L D T S L G A V O S D A G R
 AGCGTCTCACCCTCCGCAACGGCTCGCTGTGGGGGGCGGGAGCCGTGTGTGGCTGGGGCTCTCCGGGGGCGCGACCTT 320
 S V L T V R N A S L S A A C T R V E V G S E G G R T F
 CCAGCACACCGTCCGGCTCTTGTGTACGGCTTCCCGGACCACTCACCATCTCCCGGGAGCCCTGGTGGCTGGTGAAC 400
 O H T V R L L V Y A F P D O L T I S P A A L V P G D
 CGGAGGTGGCTGTACGGCCCCCAAAAGTCAGCCCTGTGGAGCCCAATCCGCTCTCTCTCTCTCTCTCTGGGGGACCA 480
 P E V A E T A H K V T P V D P N A L S F S L L L G D Q
 GAAGTGGAGGGGGCCAGCTCTGGGGCGGAGCTGGAGGAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG 560
 E L E G A Q A L G P E V E E E E E E P O E E E D V L F
 CAGGGTGACAGAGCCCTGGGGCTCCCGACCCCTGGCAACCCCTGTCTCTGGGGGGCTCTACTGCGAGGCGCAGATGAGGC 640
 R V T E R W R L P T L A T P V I P A L Y E O A T M R
 TGCTGGCTTGGAGCTCAGCCACCGCCAGGGCATCCCGCTCTGCAAGGGCCGAGCTCCCGGAGCCCGCCCGACAGGAC 720
 L P G L E L S H R D A I P V L I I G P T S R E P P D T T
 TCCCCGGAACCCCGGGCGCGGACCTCCCGGGAGACCCCGCCAGAGGGCTCCACACACACCCCGGAGGCGCGGGCTC 800
 S P E P R A A T S P E T T P Q C G S T H S P R S P G S
 TACCAGGACTTCCCGCCCTGAGATCTCCAGGCTGGGGGACCGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG 880
 T R T C R P E I S Q A G P T Q G E V I P T G S S K P
 CCGGTGACCACTGCCCCGGGGCTCTGTGGACCAAGCACTGGGGCTCTGGGAGTGGTGGCTGGCTGGCTGGCTGGCTGGCT 960
 T G D C L P A A L W T S S A V L G L L L L A I P T Y H
 CTCGGAAGAGCTTCCCGGACCTGGGTGAGGAGGGGGGGGACCCAGGCTTCTGAGTACCCAGGCTTCCCGCTGTG 1040
 L W K P C R H L A E D G A H P F A S L S S G P F P L
 AAGGAAAAATAGGTGGACCCCTTCAAGCTGAGAACGGTCCGGGGCAACCTGGCTCCCATTTCTATTCAAACCTCATCCCT 1120
 CTGTGACAGAGAGGGGACGACATTTCTCATTTGGCTCTTTGGAAAGGCTCATCAGAACTCAAAAGAGGTGATCGTTG 1200
 TCCGGCTTACCGGTGACCTGGAAAGCCCGGGCCCGGCTGGAGTGACCCCTGACTTTCTGAGGGAACCAACGTACTTETTA 1280
 CATATATTGATTCATGIGTCATATCTCCCTAAATGCTTAAAGGAGCTGTGCCCGGACCACTTGGGCCCCCTGCCATCA 1360
 GGACCTCTGAGGCTTTGGCAAAATAAACCTCCCTAAAGGATAGAAACTGAAACTTCTGCGCGCGCGCGGGTGGGTCAAGCC 1440
 TGTAAATCCAGCACTTTGGAGGGCGAGGTGGGTGSAACAGGCTCAGGAGATGAGACCATCTCTGGCTAAACCGTGA 1520
 ACCCGCTCTCTACTAAAAAAATACAAAAATTAGCCGGGAGGGGTGGCGGGCGGCTGTAGTCCAGCTACTCGGGAGGCTG 1600
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 AGAGCGAGACTCCGTCTCAAAAAAAAAAAAAAAAAAAAAA 1721

Figure 4a

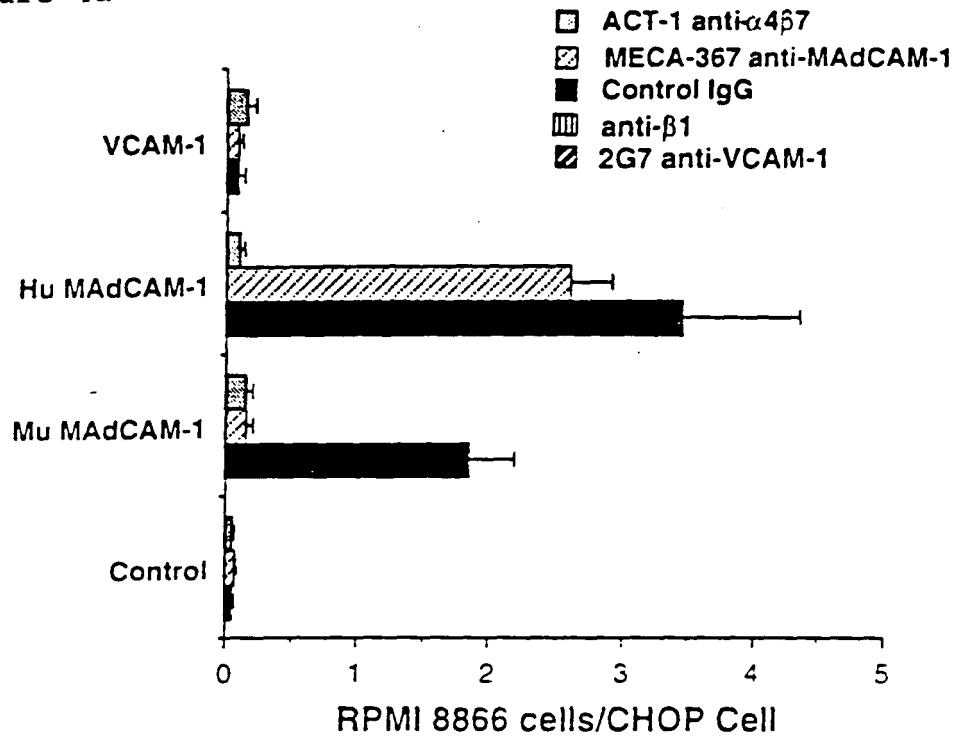
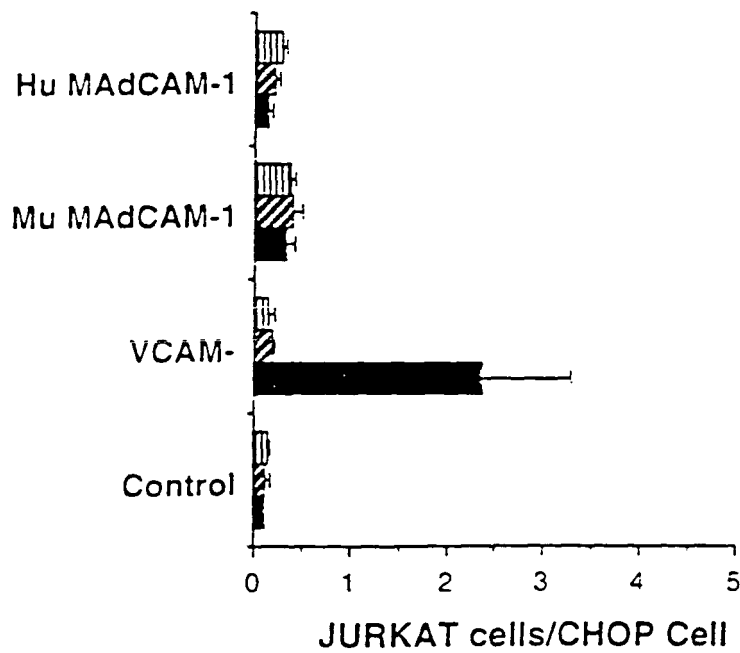


Figure 4b



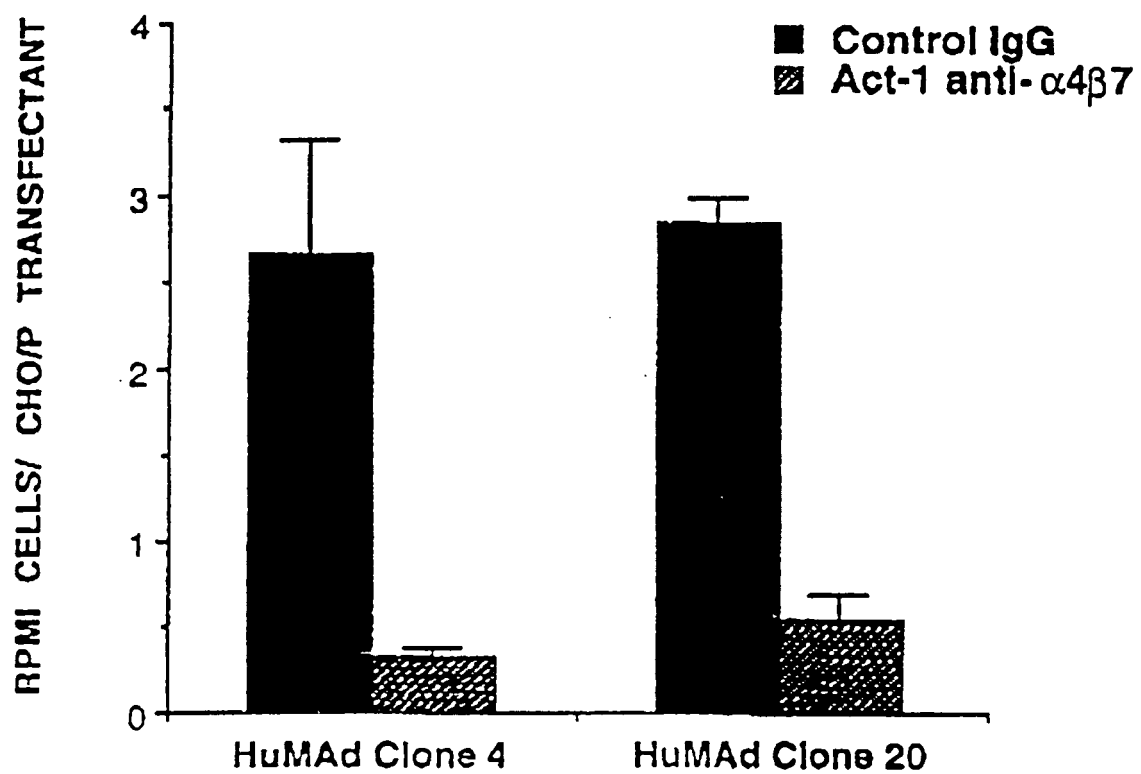


FIGURE 5

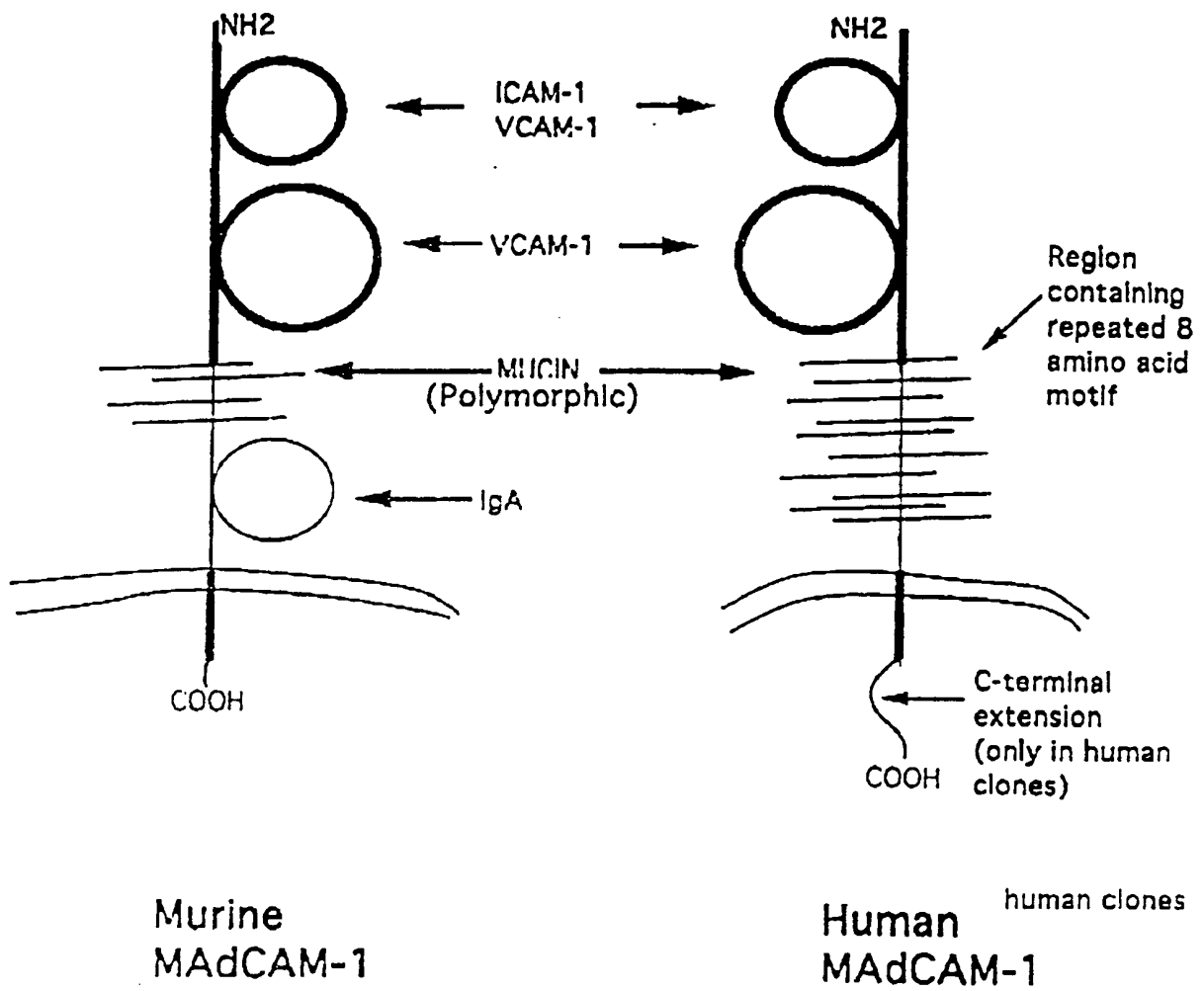


Figure 6

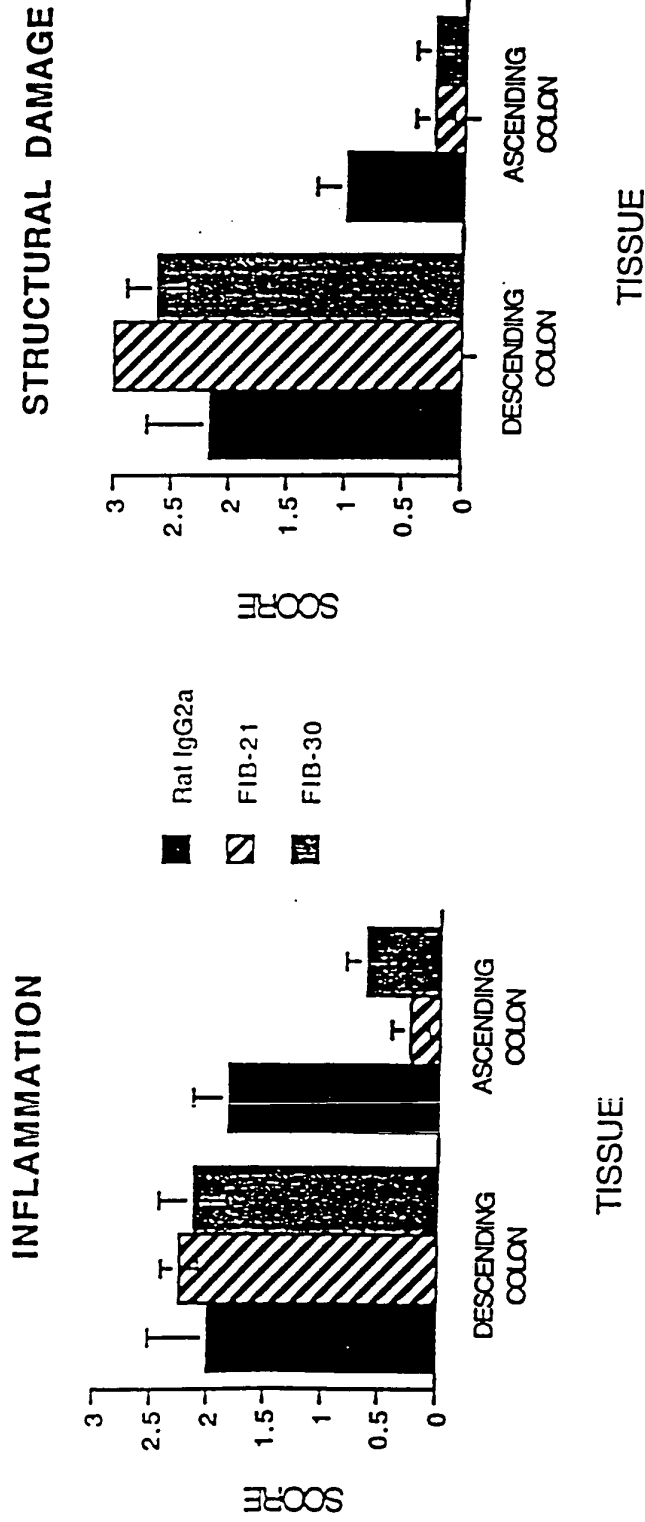
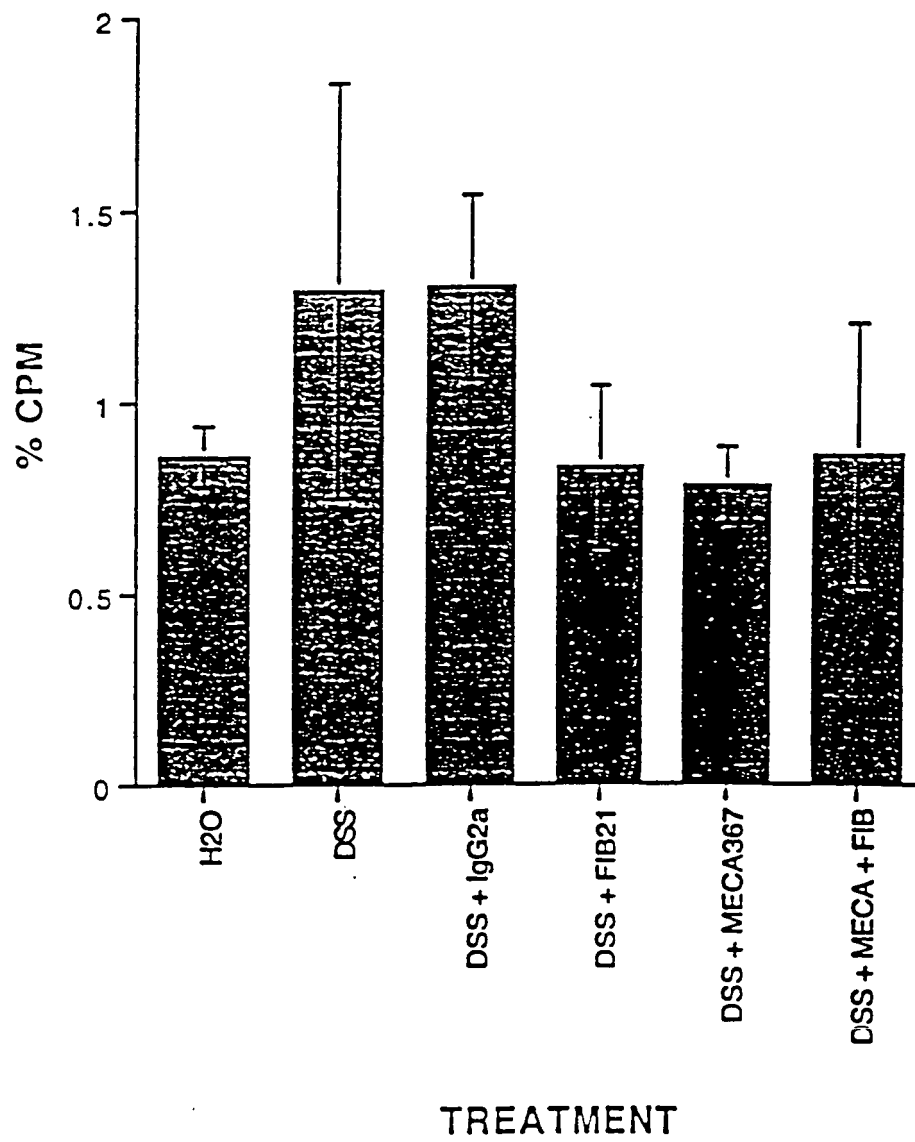


FIGURE 7A

FIGURE 7B

Figure 8



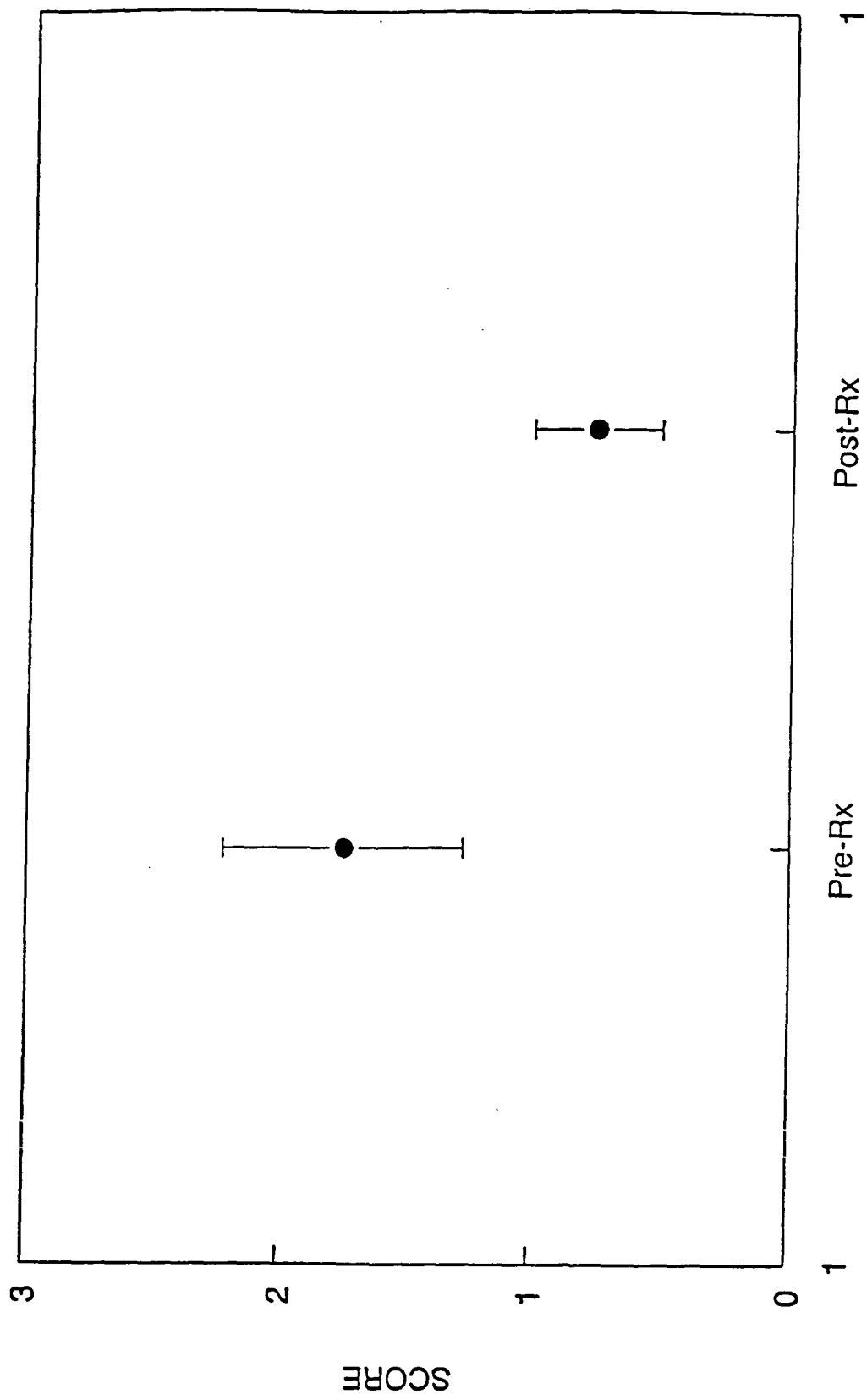


Figure 9

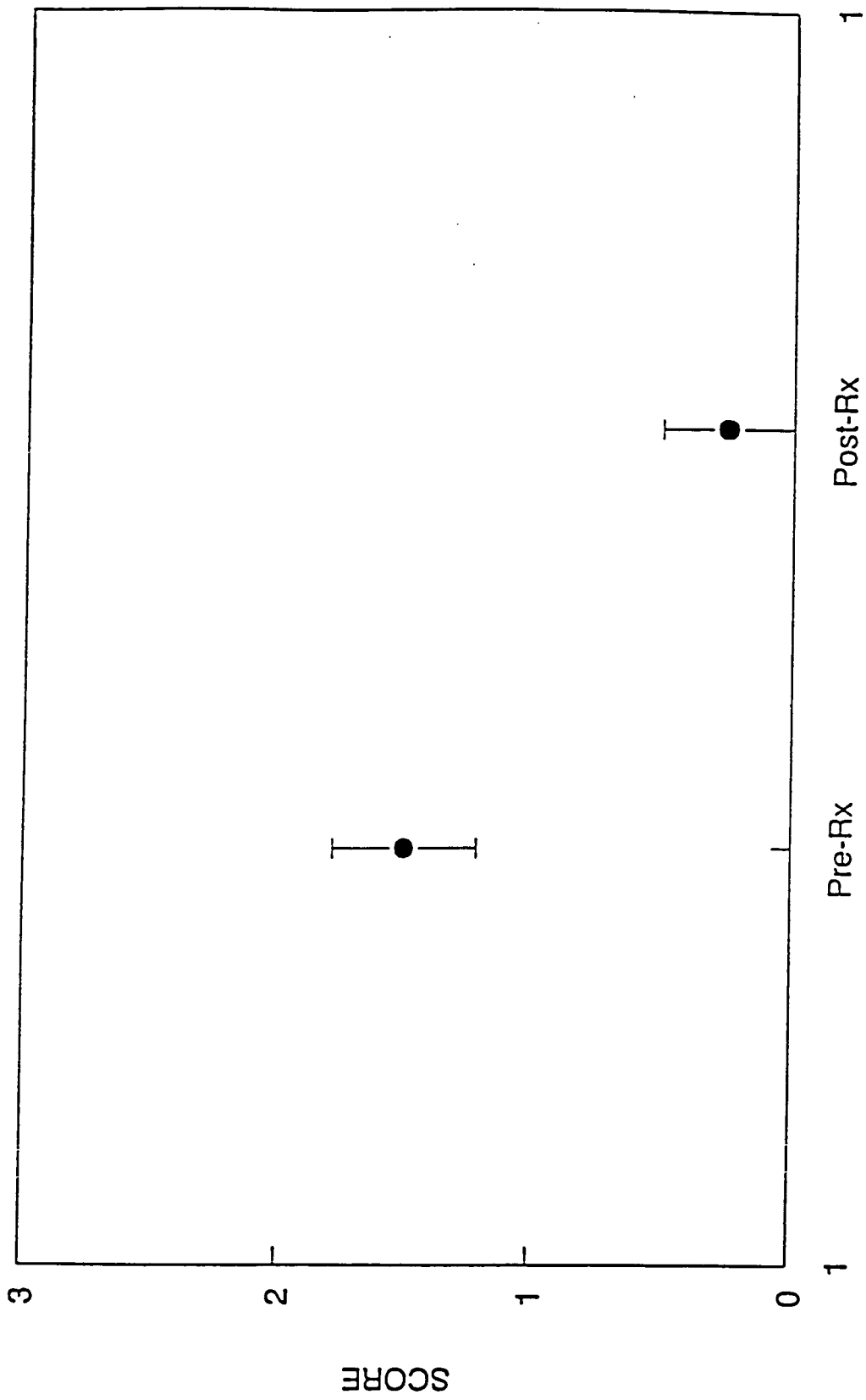


Figure 10

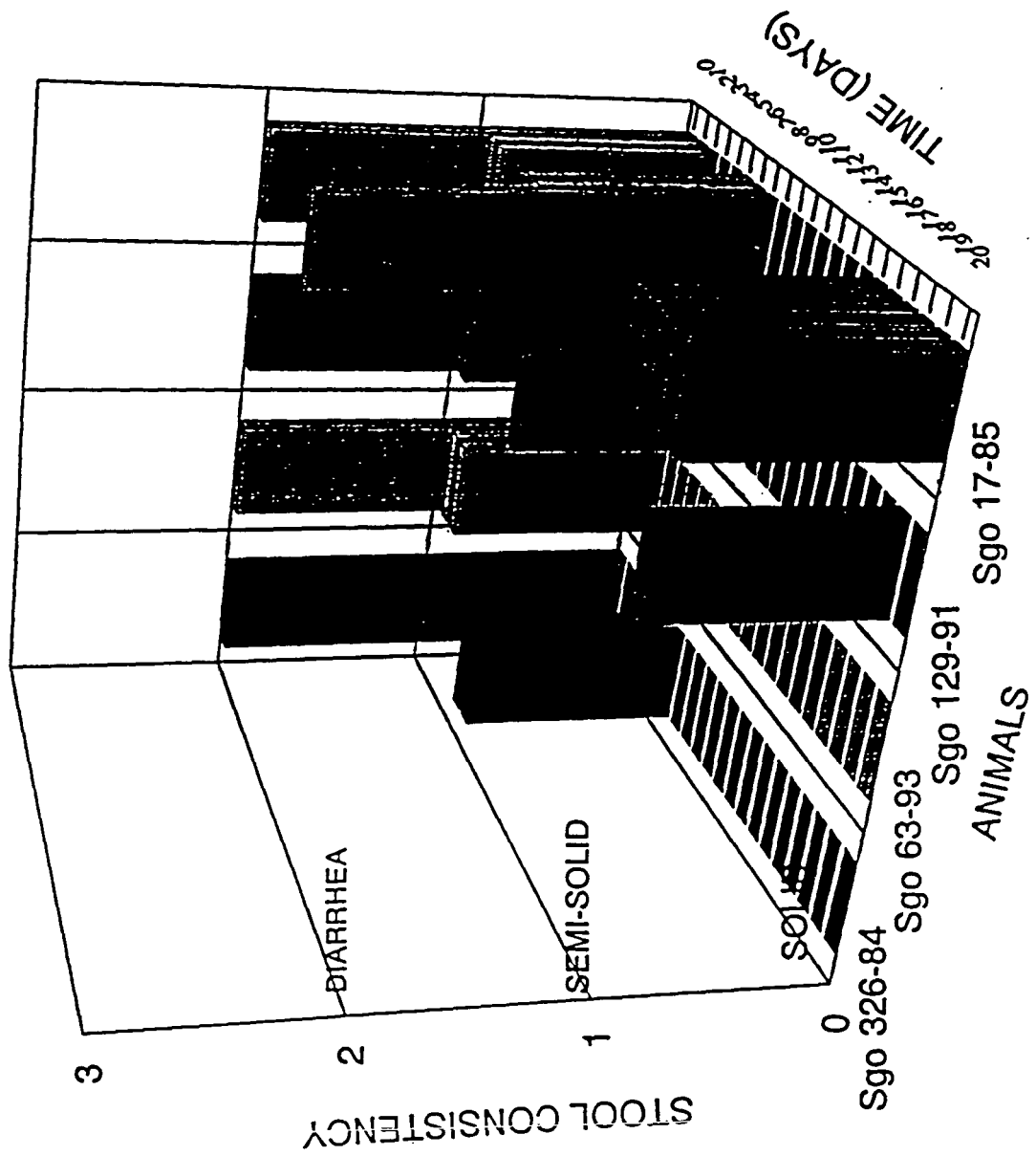


Figure 11

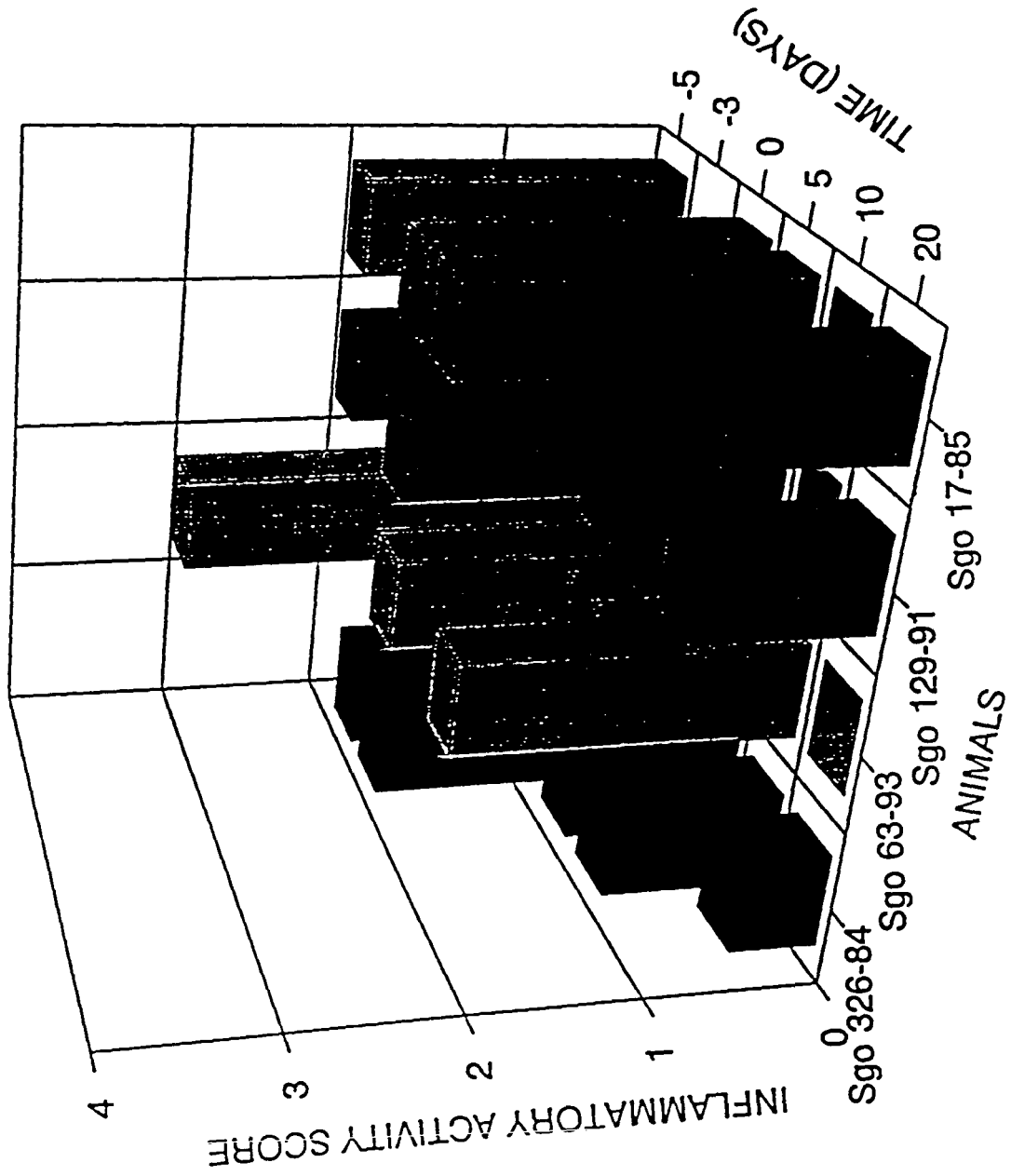


Figure 12

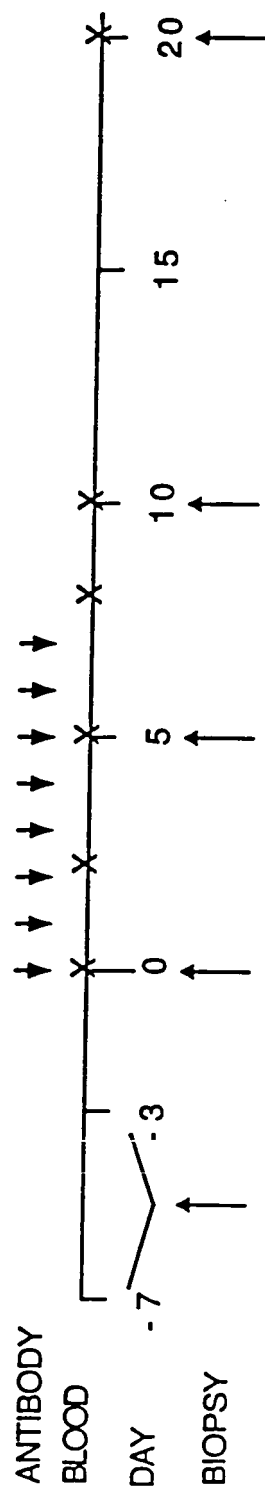


Figure 13

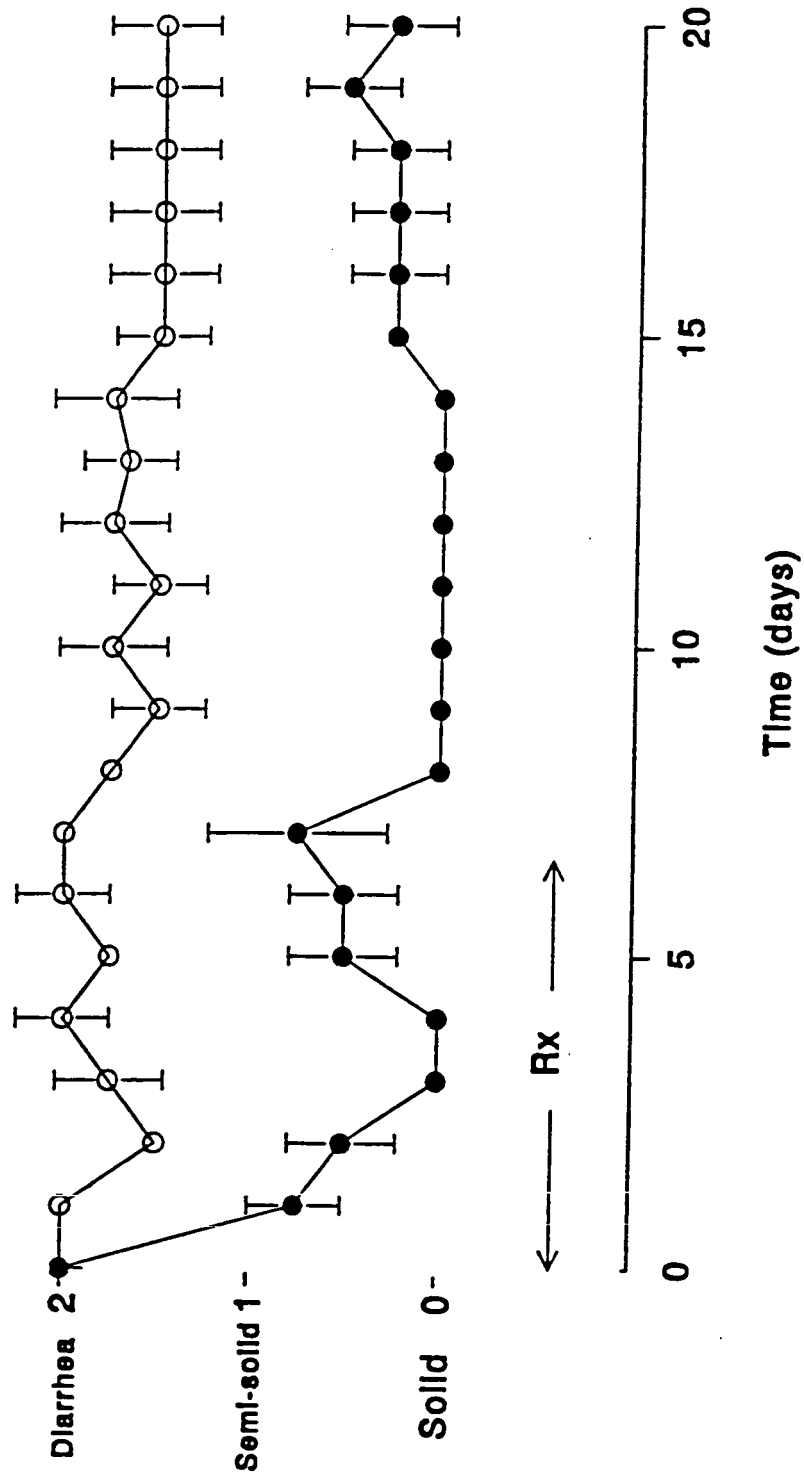
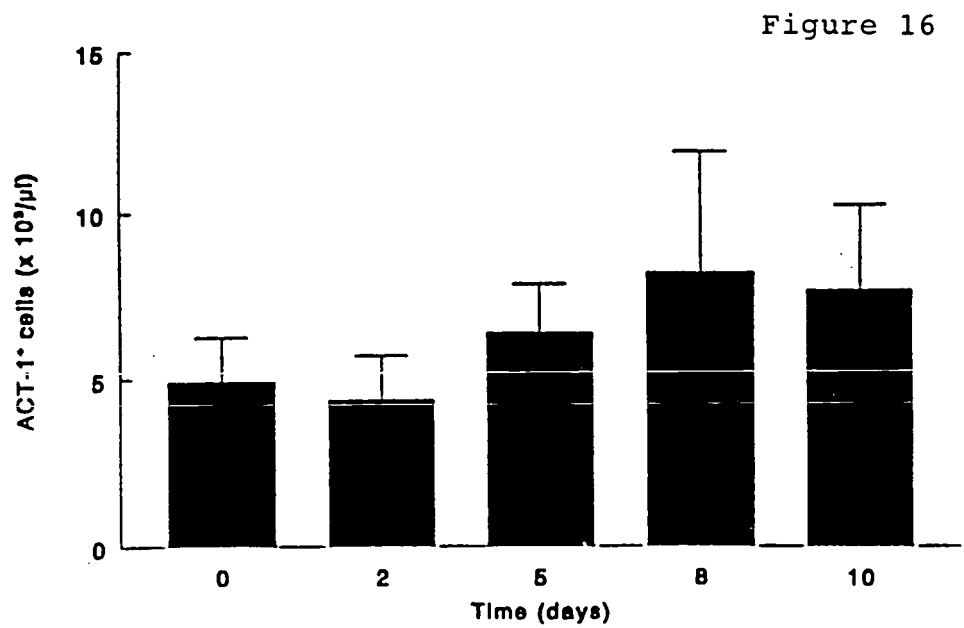
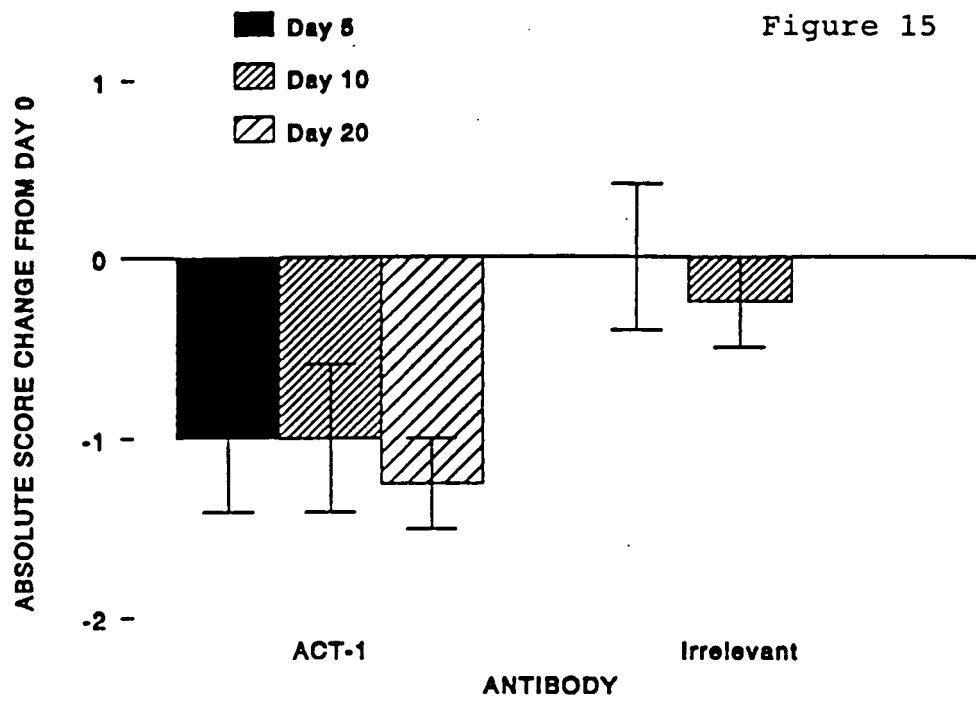


Figure 14



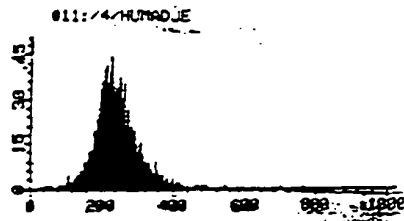


Figure 17A

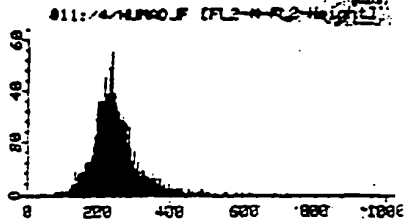


Figure 17B

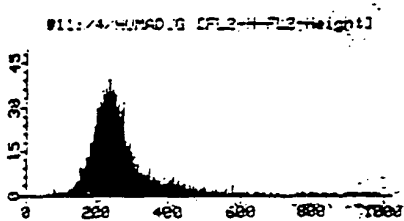


Figure 17C

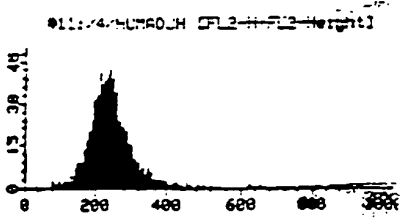


Figure 17D

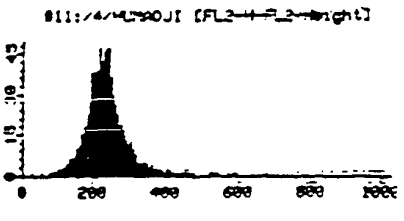
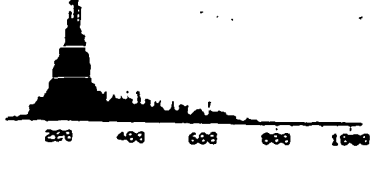
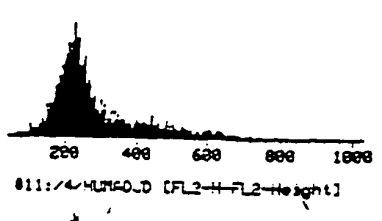
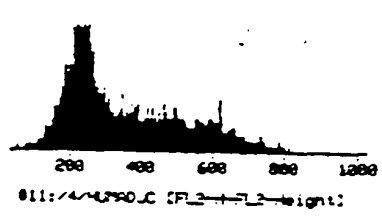
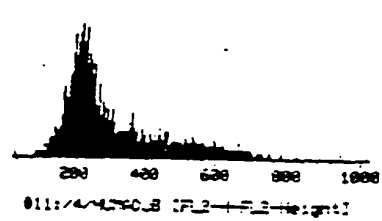
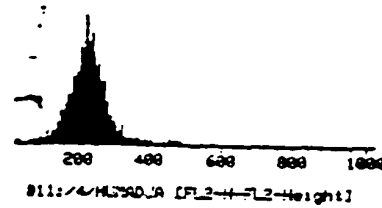


Figure 17E



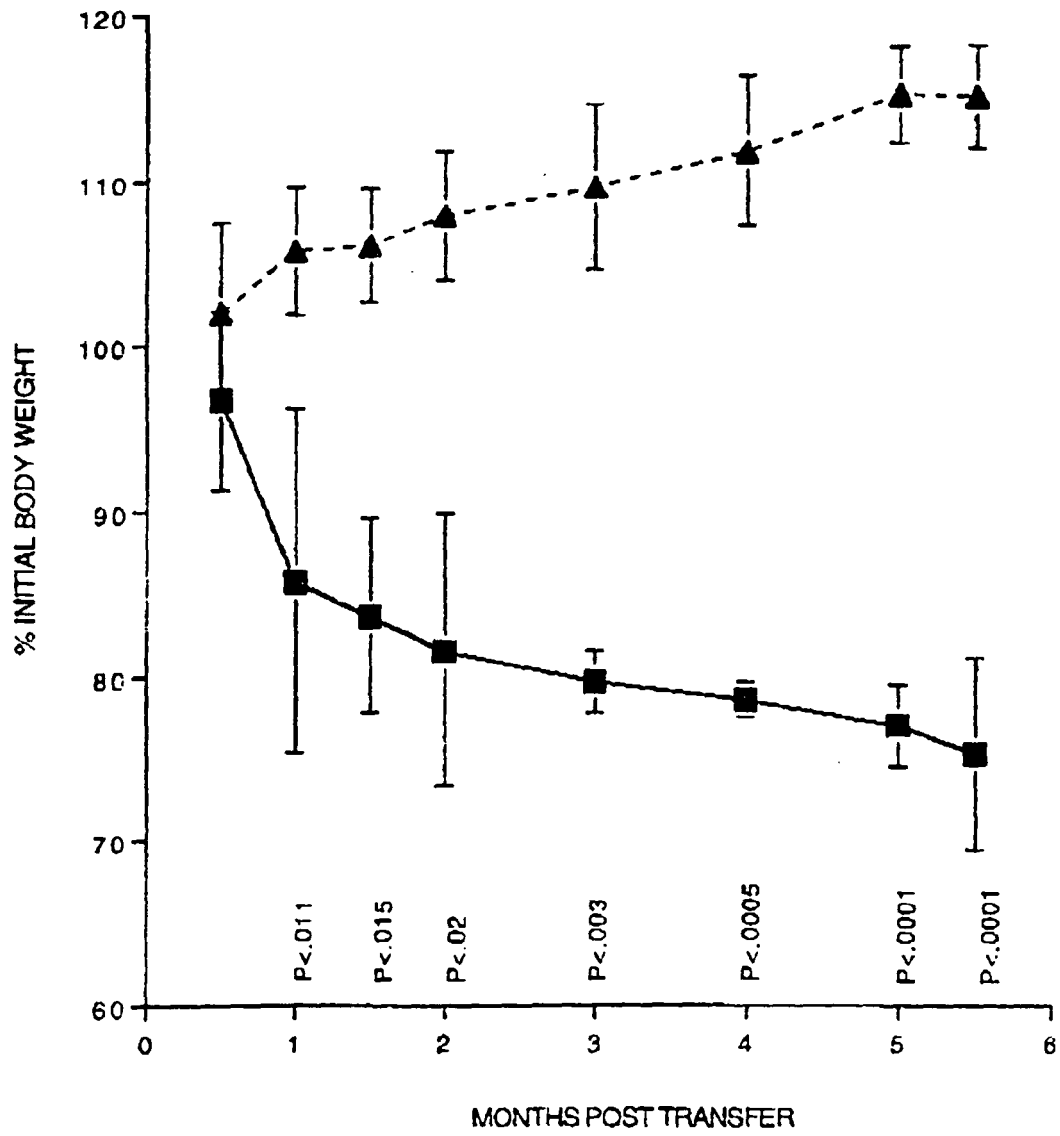


Figure 18

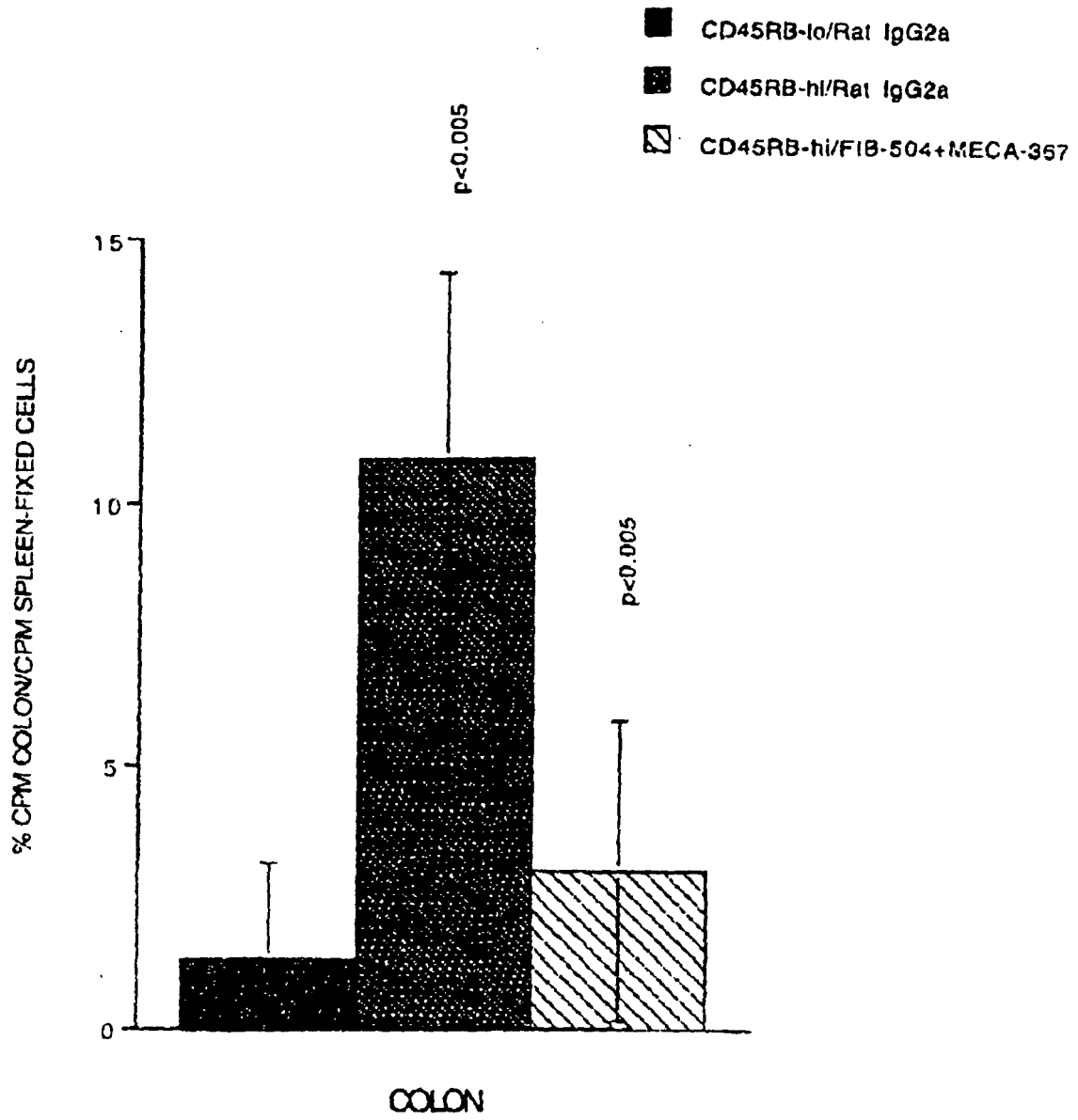


Figure 19

Figure 20

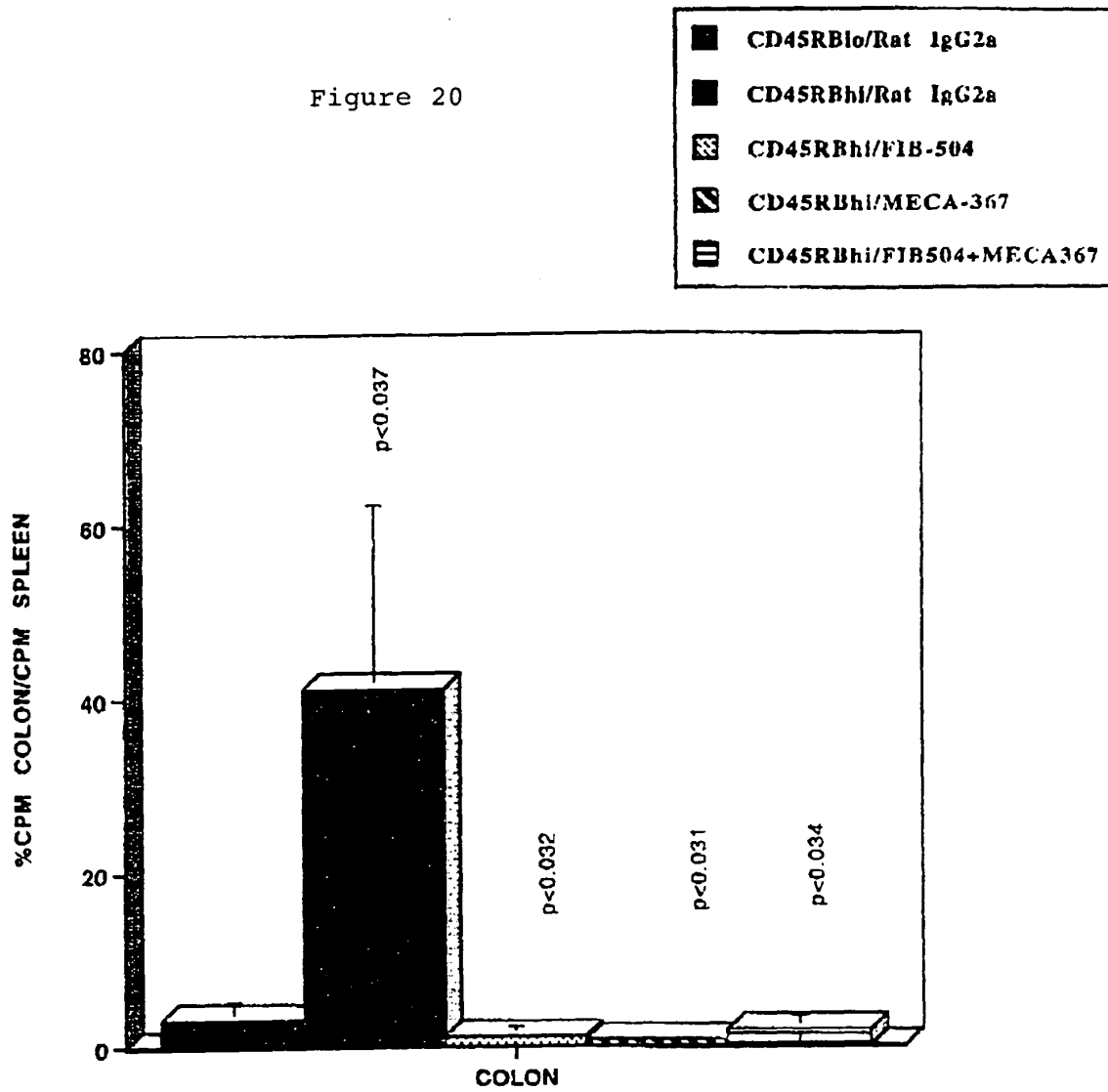
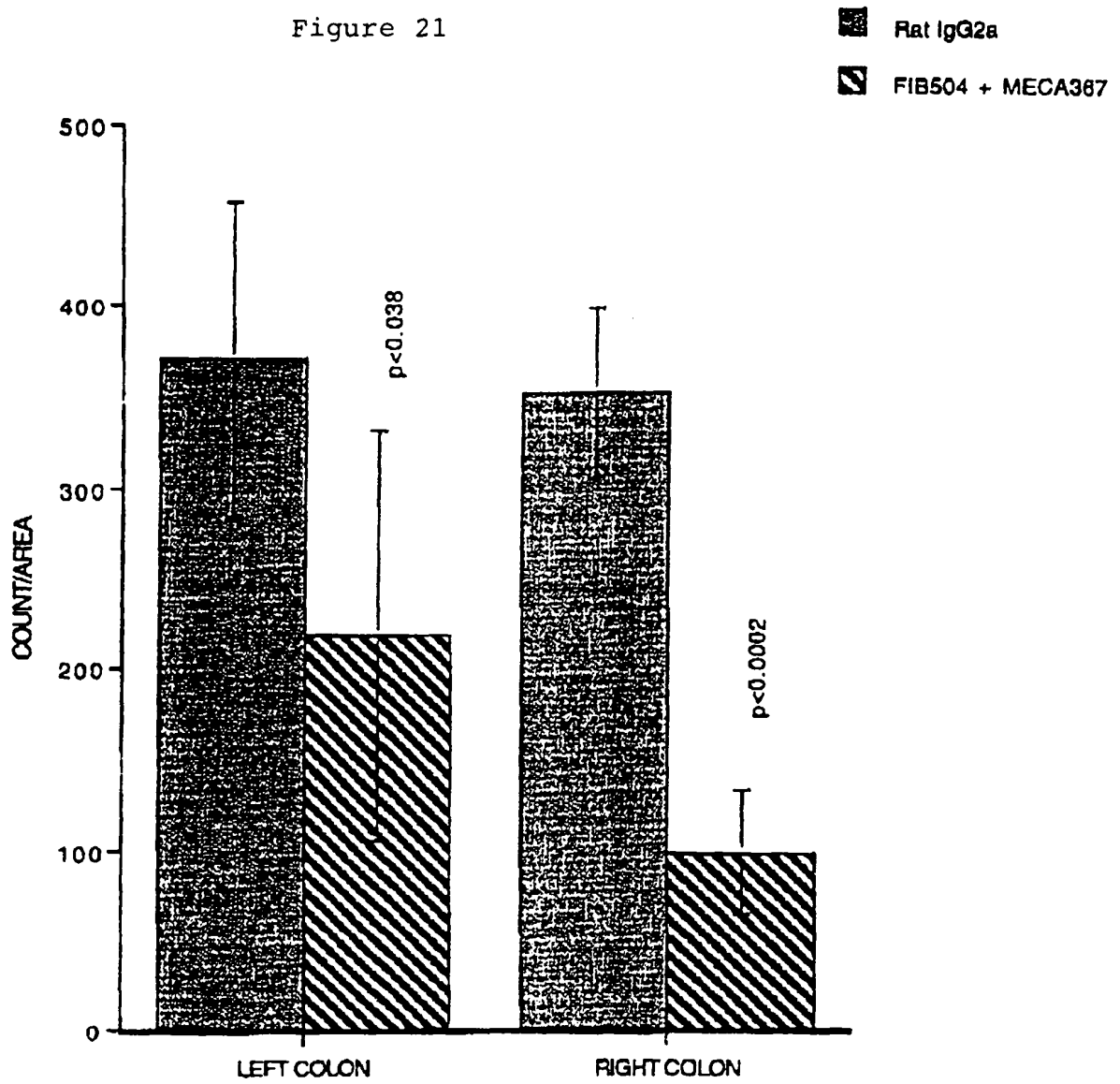


Figure 21



REFERENCES CITED IN THE DESCRIPTION

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